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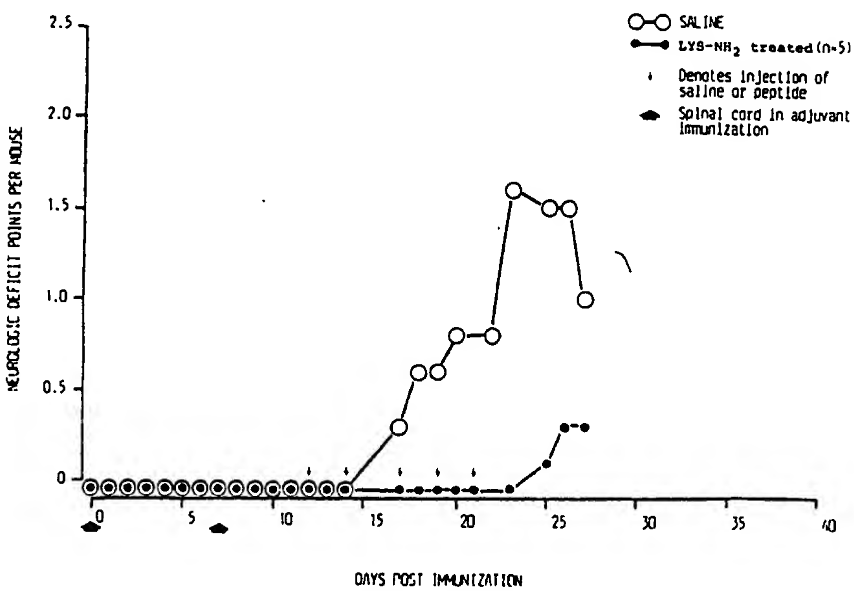
(57) Abstract

Peptides and peptide derivatives, and method of using the same in mammalian immune systems to suppress autoimmune responses, organ transplantation rejection responses, or neoplastic cell growth. The peptides are characterized by the formula A-X-(B-Y)$_n$-C wherein X and Y are residues of amino acids or amino acid derivatives with positively charged side chains, e.g., Lys, Orn, Arg, His, D-Lys, D-Orn, D-Arg, or D-His, or D-enantiomers of any of these residues, A and C are any substituents that preserve or augment the immunoregulatory activity of the peptides, B is a residue of an amino acid or amino acid derivative that preserves or augments the immunoregulatory activity of the peptide, and n is 0 or 1. The activity of the subject peptides includes suppression of the proliferation of T-lymphocytes in *in vitro* systems that are analogous to mammalian *in vivo* disease conditions, regulation of tumor cell proliferation *in vitro* and *in vivo*, and reduction of autoimmune disease-associated lesions *in vivo*. The peptides have potential human therapeutic benefits related to the treatment of autoimmune, organ or graft rejection, neoplastic and other diseases.

NEUROLOGIC DEFICIT POINTS OF SJL/J MICE IMMUNIZED WITH SPINAL CORD HOMOGENATE IN COMPLETE FREUND'S ADJUVANT AND TREATED 3 TIMES PER WEEK WITH SALINE OR 1 mg PEPTIDE LYS-NH$_2$



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IMMUNOREGULATORY PEPTIDES

BACKGROUND OF THE INVENTION

The mammalian immune system consists of a collection of cells and molecules which contribute to the defense of the host against foreign and other undesirable agents including infectious agents and cancer. The molecular portion of the immune system includes immunoglobulins, complement, histocompatibility antigens, and a variety of chemicals and peptide hormones which may either stimulate or inhibit the growth, development and activation of portions of the cellular immune system.

The cellular immune system consists of white blood cells (leukocytes) and includes lymphocytes, monocytes, macrophages, neutrophils, eosinophils, basophils and mast cells, among others. Depending on the cell type, leukocytes may circulate in blood or other body fluids, or may be fixed in various lymphoid organs including the thymus, bone marrow, lymph nodes and other organs.

In order to provide an effective immune defense, the cellular and molecular immune systems must interact in a highly intricate and complex manner. Optimally, the immune defense response should be precisely balanced to provide sufficent destructive force to eliminate infectious or foreign agents or cancer cells without unnecessarily destroying healthy cells. A suboptimal immune response may result in uncontrolled growth of an infectious agent or cancer which may lead to the death of the

host. A excessive immune response, by contrast, may destroy not only the intended target, but substantial quantities of surrounding healthy tissue as well. The many molecules of the molecular immune system play a major role in regulating and "fine tuning" the cellular immune response to maximize destruction of infectious agents and cancer cells while minimizing destruction of surrounding healthy cells.

There are many diseases which are believed to occur because of a regulatory imbalance in the immune system. Autoimmune diseases are an example of conditions in which a substantial portion of an immune response is directed toward healthy host cells.

The causes for such misdirection of immune responsiveness are unknown for many diseases. Under normal conditions, the immune system exhibits tolerance toward cells of the host which prevents the immune system from attacking normal, healthy cells. It is this critical ability of the immune system to distinguish "self" from foreign cells and molecules that provides selectivity of an immune system attack. In autoimmune diseases, tolerance for host cells and molecules is reduced or eliminated resulting in significant destruction of otherwise healthy cells and organs.

The tendency of a host's immune system to display reduced tolerance to normal cells is strongly influenced by cell surface molecules whose genes are associated with the host's major histocompatibility complex (MHC). A particular MHC haplotype may substantially increase the risk of self-tolerance loss and subsequent autoimmunity. In certain autoimmune diseases,

infection by certain viruses or bacteria is believed to trigger the loss of self-tolerance which, in the setting of an appropriate MHC haplotype, results in an autoimmune disease. In other autoimmune diseases the triggering events which lead to a loss of self-tolerance remain unknown.

Autoimmune diseases may affect every organ of the body. Examples of diseases thought to have an autoimmune pathogenesis include, but are not limited to, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, ankylosing spondylitis, Reiter's syndrome, Sjogren's syndrome, polymyositis-dermatomyositis, thrombocytopenic purpura, autoimmune hemolytic anemia, ulcerative colitis, regional enteritis (Crohn's disease), chronic active hepatitis, primary biliary cirrhosis, idiopathic interstitial pulmonary fibrosis, Goodpasture's syndrome, postviral encephalomyelitis, Guillain-Barre syndrome, myasthenia gravis, Grave's disease, Hashimoto's thyroiditis, juvenile onset insulin-dependent diabetes, Addison's disease, pernicious anemia, pemphigus, bullous pemphigoid and other diseases and conditions.

The immune response responsible for the rejection of transplanted organs among genetically non-identical animals or humans in many ways resembles an autoimmune disease in that an otherwise healthy transplanted organ may be destroyed by the

recipient's immune system. Such destruction occurs because the recipient's immune system recognizes the "foreign" histocompatibility antigens present on cells of the transplanted organ and trigger a destructive immune response.

The complex regulation of immune responsiveness results from interactions between all classes of leukocytes, molecules secreted by leukocytes and cells and molecules from other organ systems. One class of leukocytes in particular, termed thymus-derived lymphocytes or T-lymphocytes (T cells) is considered to be critically important to the coordination and regulation of most immune responses. T cells may be divided into various subsets which have distinct immune functions. Helper T cells, for example, are critical for the growth and development of B cells into antibody-secreting plasma cells. Helper T cells are also critical for the growth and development of other T cell subsets such as Killer T cells which can directly destroy infectious agents, cancer cells, transplanted organs and, in autoimmune disease, healthy cells. By contrast, other T cell subsets termed suppressor T cells actively suppress the growth and development of B cells, Killer T cells and other lymphoid cells. Suppressor T cells are also critical for the development and maintenance of immune tolerance that prevents the development of autoimmune disease and organ transplantation rejection.

Because T cells have a powerful influence on the induction or suppression of the immune responses that lead to autoimmunity and organ transplantation rejection, pharmacologic agents which regulate T cell functions may provide significant therapeutic benefit in the treatment of human or animal disease.

Thymulin and Thymic Hormone-Related Peptides

Researchers have reported peptides that exhibit effects on the immune system, including effects relating to T-lymphocyte differentiation. In 1976, Bach et al. isolated and identified from porcine thymus the nonapeptide Serum Thymic Factor (FTS), now known as thymulin, having the sequence Glx-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn (where "Glx" is either glutamine or pyroglutamic acid). (Bach, C. R. Acad. Sc. Paris, t. 283 (Nov. 29, 1976), Series D-1605; Nature 266:55 (March 3, 1977).) Thymulin has been shown to have hormonal properties related to the promotion of T-lymphocyte differentiation which include the induction of various antigenic markers on murine and human T-lymphocyte precursors, the delaying of allogeneic skin graft rejection in mice, enhancement of the generation of alloantigen reactive cytotoxic T-lymphocytes in thymectomized mice, modulation of thymic natural killer (NK) cell activity in tumor-bearing and NZB mice, inhibition of antibody-bearing lymphocyte production at late stages of differentiation, and suppression of Experimental Allergic Encephalomyelitis in guniea pigs. (Kaiserlian et al., Cellular Immunology 64:93 (1981); Kaiserlian et al., Cellular Immunology 66:360 (1982); Bardos et al., Clin. Immun. and Immunopath. 23:570 (1982); Kaiserlian et al., Clin. Immun. and Immunopath. 28:192 (1983); Immunology Today 4:16 (1983); Lenfant et al., Immunology 48:635 (1983); Nagai et al., J. Exp. Med. (Japan) 52(4):213 (1982).)

Goldstein et al. have reported in U.S. Patent Nos. 4,215,112 and 4,232,008 that both T-lymphocyte and B-lymphocyte differentiation, as measured by antigen marker induction, may be induced with tripeptides and longer peptides containing the

sequence Lys-X-Gln, where X is chosen from specified amino acid residues. The sequence of these peptides resembles that of part of the thymulin molecule, but no determination of the mechanism of activity of the peptides is stated. Imaizumi et al. concluded in 1981 that the pentapeptide portion Lys-Ser-Gln-Gly-Gly is the minimum essential part of the thymulin molecule which retains full theta-antigen induction capability for T-lymphocyte precursors. (Imaizumi et al., FEBS Letters, 128:108 (1981).) Later studies found that other thymulin segments, namely Glu-Ala-Lys-Ser-Gln and Glu-Ala-Lys-Ser, act as antagonists to thymulin activity. (Gyotoku et al., Int. J. Peptide Protein Res. 21:135 (1983).)

Goldstein et al. have described polypeptides which mimic the ability of the thymic hormones thymopoeitin and splenin to induce the differentiation of T-lymphocytes and/or B-lymphocytes. U.S. Patent No. 4,190,646 discloses the pentapeptide Arg-Lys-Asp-Val-Tyr and certain derivatives capable of inducing the differentiation of bone marrow cells to T-lymphocytes. The dipeptide Gln-Lys and certain derivatives thereof are reported in U.S. Patent No. 4,215,111 to be capable of inducing differentiation of both T- and B-lymphocytes. Other pentapeptides capable of inducing T- and/or B-lymphocyte differentiation are described in U.S. Patent Nos. 4,261,886 and 4,505,853.

A family of tetra- and pentapeptides which promote the differentiation of T-lymphocytes was described by Konig et al. in U.S. Patent No. 4,487,764. These peptides are composed of basic and aromatic amino acids in the sequence Basic-Basic-Aromatic-Aromatic-Optional.

Tuftsin and Related Peptides

The tetrapeptide tuftsin, isolated by Najjar and described in U.S. Patent No. 3,778,426, has the sequence Thr-Lys-Pro-Arg. Tuftsin has been shown to stimulate *in vitro* phagocytosis by granulocytes, monocytes and macrophages. Other studies have shown tuftsin to be active in nanomolar concentrations in many species including humans, cows, dogs, rabbits, guinea pigs and mice. In addition to its phagocytosis stimulating properties, tuftsin has been shown to stimulate such immune response functions as antibody-dependent cell-mediated cytotoxicity (ADCC) and Natural Killer cell activity in mouse splenic cells, anti-bacterial activity of PMN-leukocytes and tissue macrophages, antigen-specific macrophage-dependent T-lymphocyte education and antibody synthesis to T-lymphocyte-dependent and independent antigens *in vivo* and *in vitro*. Fridkin et al., Molecular and Cellular Biochemistry 41:73 (1981); Najjar et al., in Pick (ed.), Lymphokine Reports (Academic Press 1980), p. 157; Nishioka, Life Sciences 28:1081 (1981). A number of active analogs of tuftsin have been identified, as well as inhibitors for tuftsin such as Lys-Pro-Arg, Ala-Lys-Pro-Arg and Ser-Lys-Pro-Arg. Peptide analogs which inhi/it the immunostimulatory activity of tuftsin do not, however, reduce the basal phagocytic activity of the cells. (Najjar, Annals New York Academy of Sciences (1982), p. 1; Najjar et al., in Pick (ed.), Lymphokine Reports (Academic Press 1980), p. 157.) This suggests that immunosuppression could only be achieved via a mechanism distinct from that associated with tuftsin or its structural analogs.

Rigin, a tetrapeptide analog of tuftsin having the peptide sequence Gly-Gln-Pro-Arg, has been shown to have

phagocytosis-stimulating properties similar to those of tuftsin. (Veretennikova et al., Int. J. Peptide Protein Res., 17:430 (1981); U.S. Patent No. 4,353,823.)

SUMMARY OF THE INVENTION

It has been discovered that a number of very short peptides and peptide derivatives, including certain single-aminoacid derivatives, have a surprising ability to regulate T cell functions, and in particular to suppress T cell proliferation, and provide thereby potentially significant therapeutic benefits in the treatment of human or animal disease. Moreover, the peptides are capable of suppressing the growth of various neoplastic cells. Due to their short length, these peptides are relatively easy and inexpensive to manufacture. In addition, they are effective at non-toxic pharmacological dosage levels.

Accordingly, it is one object of the present invention to provide new peptides with important biological and pharmacological activities, as well as related and novel methods for using these peptides in suppressing the mammalian immune system *in vitro* and *in vivo*. A further object of the invention is to provide new peptides and methods related to the therapeutic treatment of mammalian autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, and other diseases.

A further object of the invention is to provide new peptides and methods related to the therapeutic treatment of diseases involving a deficient, excessive or otherwise malfunctioning mammalian immune system response, including such conditions as organ transplantation rejection and bone marrow transplantation rejection.

A still further object of the invention is to provide new peptides and methods related to the treatment of neoplastic diseases.

Other objects and advantages of the invention will become apparent from an examination of the present disclosure.

The peptides of the present invention are characterized by the formula I

$$A\text{-}X\text{-}(B\text{-}Y)_n\text{-}C \qquad \text{I}$$

wherein X and Y are residues of amino acids or amino acid derivatives with positively charged side chains, preferably lysine, ornithine, arginine, or histidine, or D-enantiomers of any of these residues; A and C are any substituents, including amino acid residues, that serve to preserve or augment the immunoregulatory activity of the peptide; B is a residue of an amino acid or amino acid derivative that preserves or augments the immunoregulatory activity of the peptide; and n is 0 or 1.

Thus, the peptides of the present invention can be characterized either by formula A-X-C (Ia) or by formula A-X-B-Y-C (Ib). Compounds of formula Ia are preferred.

Most preferably, in Ia, X will be chosen from among the amino acids lysine, ornithine, D-lysine and D-ornithine; in Ib, X will be most preferably lysine, arginine, histidine or ornithine, while Y will most preferably be lysine, arginine or histidine.

A may be, for example, hydrogen or an N-acetyl, N-acyl, N-aliphatic, or N-aromatic group; or A may be an amino acid such as alanine, glycine, leucine, valine, phenylalanine, tyrosine, asparagine, aspartic acid, sarcosine, glutamine, glutamic acid, serine, or threonine, or an amino acid derivative including D-enantiomers of these amino acids, such as D-alanine or D-aspartic acid.

B may be, for example, a residue of an amino acid such as valine, leucine, glutamic acid, glutamine, serine, threonine, glycine, alanine, proline, asparagine, aspartic acid, arginine, histidine, isoleucine, tyrosine, phenylalanine, lysine, ornithine, or tryptophan, or of a D-enantiomer or other derivative of an amino acid, such as D-valine or D-serine.

C preferably will be a small uncharged chemical constituent, for example $-NH_2$, -NHR or -OR, where R is preferably H or a lower alkyl; or C may be a residue of an amino acid such as glycine, alanine, leucine, valine, serine, cysteine, sarcosine, threonine, proline, glutamine, phenylalanine, tyrosine, homoserine or a D-enantiomer or other derivative of an amino acid.

It wil be apparent from the following disclosure that significant immunoregulatory activity will be achieved for a large number of diverse substituents in the A, B and C positions, so long as the constituents X and Y are as defined above. Thus, f.e., in Ib,

immunoregulatory activity may be retained where either or both of the terminal groups A and C comprise multiple amino acids that do not interfere substantially with the immuno-regulatory activity associated with the central tripeptide sequence X-B-Y. Accordingly, the present invention specifically contemplates that derivatives, including homologs, analogs, enantiomers, extensions, or other substitutions not specifically disclosed herein are within the scope and spirit of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

In the following discussion, the amino acid components of immunoregulatory peptides are frequently identified with abbreviations for convenience. Unless otherwise specified, the following abbreviations designate L-enantiomeric forms of the amino acids, as follows:

Amino Acid	Abbreviation
glycine	Gly
L-alanine	Ala
L-valine	Val
L-leucine	Leu
L-isoleucine	Ile
L-proline	Pro
L-methionine	Met
L-cysteine	Cys
L-phenylalanine	Phe
L-tyrosine	Tyr

L-tryptophan	Trp
L-histidine	His
L-lysine	Lys
L-arginine	Arg
L-aspartic acid	Asp
L-asparagine	Asn
L-glutamic acid	Glu
L-glutamine	Gln
L-serine	Ser
L-threonine	Thr
L-ornithine	Orn
sarcosine	Sar
L-homoserine	HomoSer

In addition, the following abbreviations for chemical substitutes are used:

Substituent	Abbreviation
N^{α}-substituted amino acid	N-R-(amino acid)
N^{α}-acylated amino acid	RCO-(amino acid)
N^{α}-acetyl amino acid	Ac-(amino acid)
N^{α}-methyl amino acid	N-Me-(amino acid)
des-α-amino acid	desamino-(amino acid)
Amino acid amide	(Amino acid)-NH_2, -NHR, -NR_2
Amino acid ester	(Amino acid)-OR.

As used herein, the term "amino acid derivative" refers to homologs, analogs, D-enantiomers, and chemically substituted or otherwise modified forms of amino acids such as those listed above. As will be seen from the following description of the invention, it has been shown that a wide range of derivatives may be substituted for naturally-occurring amino acids in the peptides of the present invention while still preserving or augmenting immunoregulatory activity. For example, chemical substitution of acyl, alkyl, and other substituents at the N-terminus, or formation of esters or amides at the C-terminus, will frequently yield peptide derivatives with preserved or augmented immunoregulatory activity.

Furthermore, the present invention contemplates that certain amino acids within the disclosed peptides may be substituted by amino acids which are chemically similar by virtue of similar side chain size, charge, shape, solubility, or other chemical characteristics while still retaining the peptide's immunoregulatory activity. Amino acids with such chemical similarity are termed "functionally conserved." Functional classes of the common genetically-coded amino acids have been specified by Dayhoff, et al. in Atlas of Protein Sequence and Structure, volume 5 (National Biomedical Research Foundation, 1972), page 98. Derivatives of the naturally-occurring amino acids, such as ornithine, homoserine, homolysine, des-N^{α}-amino lysine and homoarginine, have chemical structures and properties comparable to their naturally-occurring analogs or homologs, and

thus may, in proper cases, be substituted in the peptides of the present invention to preserve or even increase immunoregulatory activity.

One aspect of the immunoregulatory activity of the subject peptides resides in the capability of the peptides to suppress autoimmune responses, to suppress organ transplantation rejection responses, or to suppress neoplastic cell growth. As will be discussed below, such immunoregulatory activity may be realized in _in vitro_ systems that are modeled after _in vivo_ mammalian disease conditions, as well as in _in vivo_ systems.

The immunoregulatory peptides of the present invention are characterized by the formula

$$A\text{-}X\text{-}(B\text{-}Y)_n\text{-}C \qquad \text{I}$$

wherein

X and Y are residues of amino acids or amino acid derivatives with positively charged side chains at physiological pH (i.e., pH 6 to 8) or D-enantiomers of any of these residues; A and C are amino acid residues or other substituents, that preserve or augment the immunoregulatory activity of the peptide; B is a residue of an amino acid or amino acid derivative that preserves or augments the immunoregulatory activity of the peptide; and n is 0 or 1.

Thus, in Ia, X may be chosen from among residues of such naturally-occurring positively charged amino acids as Lys, Arg and His.

In addition, however, it has been discovered that residues of positively charged amino acids other than those naturally-occurring acids listed above may be used successfully in the X position. For example, substitution of the D-enantiomeric form of the amino acid X will, in some cases, augment, and, in many cases, substantially preserve the immunoregulatory activity of the subject peptide. As another example, residues of desamino forms of the central amino acid X, wherein the N^{α}-amino group of X is absent, may preserve or augment the immunoregulatory activity of the peptide.

Other amino acid derivatives, including homologs, analogs, enantiomers and otherwise modified forms of naturally occurring positively charged amino acids, can also be expected to yield significant immunoregulatory activity. As an example, ornithine, which is closely homologous to lysine, demonstrates significant activity despite the fact the ornithine does not occur naturally in proteins or in other molecular constituents of the immune system.

Especially preferred for X are Lys and Orn. The D-enantiomeric form of these amino acids (D-Lys, D-Orn) as well as the deaminated forms (desamino-Lys, desamino-Orn) may be substituted for the naturally occurring L-enantiomer

in proper cases, with the result that immunoregulatory activity or the peptide is substantially preserved or augmented.

In Ib, the structure of the three central constituents of the subject polypeptides is of central importance. In its simplest from, Ib countenances a tripeptide of the form

X-B-Y

wherein X and Y are any positively-charged amino acids separated by any single amino acid, B, which tripeptide has immunoregulatory activity. In this form of the present invention, terminal constituent A is hydrogen and terminal constituent C is a hydroxyl group. X and Y may be the same or different positively charged amino acids, preferably chosen from among Lys, Arg, His and Orn.

Lys and Arg are particularly preferred in the X and Y positions. Thus, the following tripeptide sequences are particularly preferred in the present invention:

Lys-B-Lys

Arg-B-Arg

Lys-B-Arg

Arg-B-Lys

In addition to the tripeptide sequences given above, immunoregulatory activity may be achieved through the use of

His or Orn in the X or Y positions. His or Orn may be substituted in the X position, while His is preferred in the Y position.

In addition to the common forms of the amino acids discussed above, the present invention contemplates that modified, homologous, analogous or artificial forms of positively charged amino acids may be utilized in the X and Y positions of Ib. Thus, D-enantiomeric forms of positively charged amino acids may, in appropriate combination with other constituents of the subject polypeptides, substantially preserve or augment the immunoregulatory activity of the peptide.

Constituent B comprises the residue of any amino acid or amino acid derivative which preserves or augments the immunoregulatory activity of the subject polypeptides. It has been discovered that a very broad range of amino acids may be incorporated into the B position to yield polypeptides with significant immunoregulatory activity. Preferably, B will be chosen from among the residues of the following amino acids or their D-enantiomers: Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Pro, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp.

Most preferably, B will be chosen from among the following amino acids or their D-enantiomers: Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala.

As discussed above, the B position amino acids listed are preferably used in conjunction with the positively charged amino acids Lys or Arg in the X und Y positions. In peptides in which both X and Y are Lys, the amino acids Val, Glu and Gln are especially preferred in the B position. When both X and Y are Arg, Asp and Ser are highly active substituents in the B position. Ser and Thr are examples of suitable B substituents when X is Lys and Y is Arg, while Gly, Ala, Ser and Thr are all active when X is Arg and Y is Lys.

The terminal constituent A, which is substituted at the N^{α}-position of X, may be chosen from a number of chemical groups or substituents. For example, terminal constituent A may be hydrogen (H-) alone, yielding a peptide of the general formula $X-(B-Y)_n-C$.
As another example, constituent A may be an N^{α}-acetyl substituent, thus yielding a peptide of the general formula

$$Ac-X-(B-Y)_n-C.$$

Other substituents in the A position that may preserve or augment immunoregulatory activity include aliphatic and aromatic acyl substituents of the form RCO-, as well as substituents of the form R-, where R is preferably an unbranched or branched alkyl group of one to eight carbons, and may also be C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_6-C_{14} aryl, C_7-C_{14} alkaryl, C_7-C_{14} aralkyl, or C_3-C_{14} cycloalkyl.

Furthermore, constituent A may be an amino acid. Ala and D-Ala are especially preferred amino acids for constituent A. Also preferred as amino acids for terminal constituent A are the following: Gly, Leu, Val, Phe, Tyr, Asn, Asp, Sar, Gln, Glu, Ser and Thr as well as the D-enantiomers of these amino acids.

In addition to single amino acid substitutions in the A terminal position, substitutions of more than one amino acid have been shown to yield active polypeptides, particularly of formula Ib. In particular, A terminal constituents of the form Val-Asp-, Gly-Asp-, Ala-Asp-, Ser-Asp-, Thr-Val-Asp- and Leu-Thr-Val-Asp- have been successfully used in the A position. Thus, it is believed that the immunoregulatory activity of the present polypeptides may be preserved with a large number of diverse substituents, including sequences containing multiple amino acids, in the A position as well as in the C terminal position.

It will be apparent to those skilled in the art that modifications, additions, deletions, or substitution to the chemical groups named above as A terminal constituents may preserve activity without departing from the spirit of the invention described herein.

Terminal constituent C also may be chosen from a number of chemical groups or substituents which preserve or augment the immunoregulatory activity of the subject

peptides. In general, it is preferred that C be a relatively small, uncharged species (including a hydroxyl (-OH) group). Furthermore, amidation, esterification and addition of one or more amino acids is possible in the terminal group C. For example, one preferred embodiment of C is of the form

-NHR

wherein R is hydrogen or a lower alkyl, such as C_1-C_8 alkyl substituent. As an example of the latter, wherein R is ethyl, and where X is Lys and A is hydrogen, Lys-$NHCH_2CH_3$ is representative.

Other substituents in the C terminal position may preserve or augment immunoregulatory activity, most preferably relatively small and uncharged species. Thus, R groups such as C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_6-C_{14} aryl, C_7-C_{14} alkaryl, C_7-C_{14} aralkyl and C_3-C_{14} cycloalkyl may be utilized in C terminal substituents of the form -NHR or -OR to yield amides and esters, respectively. Secondary amino groups of the form $-NR_2$ may also be expected to yield active peptide amides.

Particularly significant immunoregulatory activity may be obtained by using lower alkyl R groups in the polypeptide amides described above. C_1-C_3 lower alkyl groups are particularly preferred. In particular, significant activity has been obtained in tripeptide amides of the form

Lys-B-Lys-NH_2

wherein B is Val, Leu, Pro, Glu, Gln or Gly. Contrarily, amidation where the B position amino acid is Asp, Ala, Thr, Arg, or His yields peptides with lower, although still significant, immunoregulatory activity.

Amino acids and derivatives thereof may also be used as C terminal constituents. In general, it is particularly preferred that such amino acids have relatively small, uncharged side chains. Thus, Ser, Gly, Ala, Leu, Val and Gys, are particularly preferred as C terminal constituents.

Ser has been shown to yield a particularly high immunoregulatory activity, particularly in the combination Lys-Ser. Gly and Ala are especially preferred in the combinations Orn-Gly, Lys-Gly and Orn-Ala.

As with the examples discussed above with respect to amino acid constituents at the A and X positions, the D-enantiomeric form of amino acids in the C terminal position may demonstrate significant immunoregulatory activity.

The combination Orn-D-Ala is especially preferred.

Although amino acids having relatively small, uncharged side chains are particularly preferred as C constituents in the present invention, significant immunoregulatory activity is also achieved using amino acids with relatively larger uncharged side chains as C terminal constituents. Also preferred, therefore, as C terminal constituents are the following amino acids, as well as their D-enantiomers: Thr, Pro, Gln, Phe, Tyr.

Other amino acids, or derivatives thereof, including homologs, analogs, enantiomers, or combinations thereof, as well as other non-amino acid chemical constituents, may likewise be incorporated into the C terminal position to substantially preserve or augment the immunoregulatory activity of the subject peptides. For example, HomoSer and Sar respectively, may be utilized successfully in the present invention as C terminal constituents.

In addition, amides or esters of C terminal amino acids may be so used. The peptide Lys-Ser-NH_2 is particularly preferred. Furthermore, combinations or sequences of amino acids which substantially preserve or augment the immunoregulatory activity of the subject peptides may be incorporated as C terminal constituents. It is believed that it is within the skill of the applicable art to identify and utilize as C terminal constituents those chemical substituents, including amino acids and their derivatives, that substantially preserve or augment the immunoregulatory activity of the subject peptides, and which are not disclosed specifically herein.

It is apparent from the foregoing discussion that a peptide of the present invention may incorporate one, two, three, or more amino acids. In its simplest form, the present invention contemplates a single, positively charged amino acid (the central amino acid, X) flanked by non-amino acid terminal constituents A

and C. Certain of these "monopeptides" demonstrate extremely high immunoregulatory activity. Particularly preferred are lower alkyl amides of Lys, Orn, D-Lys and D-Orn.

Thus, the following monopeptides are particularly preferred:

Lys-NH_2

Lys-$NHCH_3$

Lys-$NHCH_2CH_3$

D-Lys-NH_2

D-Lys-$NHCH_3$

D-Lys-$NHCH_2CH_3$

Orn-NH_2

Orn-$NHCH_3$

Orn-$NHCH_2CH_3$

D-Orn-NH_2

D-Orn-$NHCH_3$

D-Orn-$NHCH_2CH_3$

The present invention countenances a large number of highly active dipeptides. Many of these are characterized by an A terminal constituent that comprises a non-amino acid species and a C terminal constituent that comprises an amino acid or a homolog, analog, or other derivative thereof. In particular, it is particularly preferred that the C terminal constituent be Ser, Lys, Ala or Cys, and that the central amino acid X be Lys or Orn. The A terminal constituent is preferably hydrogen (H-) or an acetyl group.

In addition, the C terminal constituent may be amidated to form a lower alkyl amide. Thus, dipeptides of the following general form are particularly preferred:

X-C

$X-C-NH_2$

$X-C-NHCH_3$

$X-C-NHCH_2CH_3$

Ac-X-C

$Ac-X-C-NH_2$

$Ac-X-C-NHCH_3$

$Ac-X-C-NHCH_2CH_3$

wherein C is preferably Ser, Gly, Ala, or Cys, and X is preferably Lys or Orn. The combinations **Lys-Ser, Lys-Gly, Lys-Ser-NH_2, Orn-Gly and Orn-Ala are especially preferred.**

The use of D-enantiomeric forms of amino acids in the X and C positions has been shown to substantially preserve or augment immunoregulatory activity in a number of cases. Particularly preferred D-enantiomeric substitutions include, in the central amino acid X position, D-lysine and D-ornithine, and, in the C terminal position, D-alanine. D-Lys-Ser and Orn-D-Ala have been shown to have particularly high immunoregulatory activity.

In addition to the foregoing class of dipeptides, in which the A terminal constituent is not an amino acid, the present invention contemplates that class of dipeptides characterized by an amino acid A terminal constituent in conjunction with a non-amino acid C terminal constituent. In general, for Ia, it is preferred that the C terminal constituent be a group other than a hydroxyl group (-OH), although dipeptides such as Asp-Lys and Ser-Lys, in which C is a hydroxyl group, have been shown to exhibit immunoregulatory activity. A C terminal substituent of the form -NHR, wherein R is preferably hydrogen or a lower alkyl, accompanied by an A-position amino acid as discussed below, will yield a dipeptide amide of the central positively charged amino acid X. Amides of this central amino acid have been shown to have particularly significant immunoregulatory activity.

Particularly preferred as A terminal amino acids are Ala, Gly, Val, Phe, Tyr, Asp and Sar, as well as the D-enantiomers of these amino acids. Ala is especially preferred as an A terminal constituent in peptides of the form

Ala-X-C

wherein X is preferably Lys, D-Lys, Orn, or D-Orn, and C is preferably Gly, Ala, D-Ala, or Ser. In addition the use of D-Ala as an A terminal constituent is preferred, yielding, f.e. a peptide of the form

D-Ala-X-C,

wherein X and C are preferably as defined immediately above.

Modifications of the C terminal constituent, as for example, by amidation to a lower alkyl amide, may also yield peptides having potent immunoregulating activity. Other modifications to the constituents of the subject peptides will be recognized by those skilled in the art and can readily be synthesized, tested and utilized within the scope of the present invention as described herein.

Specifically preferred are peptides of formulae Iaa to Iam, Iama to Iamz and Ian to Iat which correspond to formula Ia, but wherein

in Iaa: X is a residue of an amino acid or amino acid derivative with a positively charged side chain;

A is H, R, RCO, or a residue of an amino acid or amino acid derivative;

C is NH_2, NHR, NR_2, OR, or a residue of an amino acid or amino acid amide or ester formed by substitution of an amino acid with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Iab: X is a residue of an amino acid or amino acid derivative with a positively charged side chain;

A is H, acetyl, Ala, Gly, Val, Phe, Tyr, Asp, Sar, Ser, Thr, D-Ala, D-Val, D-Phe, D-Tyr, D-Asp, D-Ser or D-Thr; and

C is any chemical substituent, residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

in Iac: X is a residue of an amino acid or amino acid derivative with a positively charged side chain;

A is any chemical substituent, residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide; and

C is NH_2, NHR, OR, Gly, Ala, Ser, Cys, Sar, Thr, Pro, Gln, Phe, Tyr, HomoSer, D-Ala, D-Ser, D-Cys, D-Thr, D-Pro, D-Gln, D-Phe, D-Tyr, D-HomoSer, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR or OR, where R is a C_1-C_8 alkyl;

in Iad: X is a residue of an amino acid or amino acid derivative with a positively charged side chain;

A is H, R, RCO, Ala, Gly, Val, Phe, Tyr, Asp, Sar, Ser, Thr, D-Ala, D-Val, D-Phe, D-Tyr, D-Asp, D-Ser or D-Thr;

C is NH_2, NHR, OR, Gly, Ala, Ser, Cys, Sar, Thr, Pro, Gln, Phe, Tyr, HomoSer, D-Ala, D-Ser, D-Cys, D-Thr, D-Pro, D-Gln, D-Phe, D-Tyr, D-HomoSer, or a residue of an amino acid amide or est er formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, or OR; and

R is a C_1-C_8 alkyl;

in Iae: X is a residue of an amino acid or amino acid derivative with a positively charged side chain;

A is H, acetyl, Ala, and D-Ala; and

C is NHR, Gly, Ala, Ser, D-Ala, D-Ser, Gly-NHR, Ala-NHR, Ser-NHR, D-Ala-NHR, or D-Ser-NHR, where R is a C_1-C_8 alkyl;

in Iaf: X is Lys, Orn, Arg, His, D-Lys, D-Orn, D-Arg, or D-His;

A is any chemical substituent, residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide; and

C is any chemical substituent, residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

In Iag: X is Lys, Orn, Arg, His, D-Lys, D-Orn, D-Art or D-His;

A is H, R, RCO, a residue of an amino acid or an amino acid derivative;

C is NH_2, NHR, NR_2, -OR, or a residue of an amino acid or amino acid amide or ester formed by substitution of an amino acid with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Iah: X is Lys, Orn, Arg, His, D-Lys, D-Orn, D-Arg, or D-His;

A is H, R, RCO, Ala, Gly, Val, Phe, Tyr, Asp, Sar, Ser, Thr, D-Ala, D-Val, D-Phe, D-Tyr, D-Asp, D-Ser and D-Thr;

C is NH_2, NHR, OR, Gly, Ala, Ser, Cys, Sar, Thr, Pro, Gln, Phe, Tyr, HomoSer, D-Gly, D-Ala, D-Ser, D-Cys, D-Ser, D-Thr, D-Pro, D-Gln, D-Phe, D-Tyr, D-HomoSer, or a residue of an amino acid amide and or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, or OR; and

R is a C_1-C_8 lower alkyl;

in Iai: X is Lys, Orn, Arg, His, D-Lys, D-Orn, D-Arg, or D-His;

A is H, acetyl, Ala, or D-Ala; and

C is NHR, Gly, Ala, Ser, D-Ala, D-Ser, Gly-NHR, Ala-NHR, Ser-NHR, D-Ala-NHR, or D-Ser-NHR, where R is a C_1-C_8 alkyl;

in Iaj: X is Lys, Orn, D-Lys or D-Orn;

A is any chemical substituent, residue of an amino acid, or amino acid derivative that preserves the immunoregulatory activity of said peptide; and

C is any chemical substituent, residue of an amino acid, or amino acid derivative that preserves the immunoregulatory activity of said peptide;

in Iak: X is Lys, Orn, D-Lys or D-Orn;

A is H, R, RCO, or a residue of an amino acid or amino acid derivative;

C is NH_2, NHR, NR_2, OR, or a residue of an amino acid or amino acid amide or ester formed by substitution of an amino acid with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ial: X is Lys, Orn, D-Lys or D-Orn;

A is H, R, RCO, Ala, Gly, Val, Phe, Tyr, Asp, Sar, Ser, Thr, D-Ala, D-Val, D-Phe, D-Tyr, D-Asp, D-Ser or D-Thr; and

C is NH_2, NHR, Gly, Ala, Ser, Cys, Sar, Thr, Pro, Gln, Phe, Tyr, HomoSer, D-Ala, D-Ser, D-Cys, D-Thr, D-Pro, D-Gln, D-Phe, D-Tyr, D-HomoSer, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, or OR; and

R is a C_1-C_8 alkyl;

in Iam: X is Lys, Orn, D-Lys or D-Orn;

A is H, acetyl, Ala, or D-Ala; and

C is NHR, Gly, Ala, Ser, D-Ala, D-Ser, Gly-NHR, Ala-NHR, Ser-NHR, D-Ala-NHR, or D-Ser-NHR, where R is C_1-C_8 alkyl;

in Iama: X is Lys or D-Lys;
A is H; and
C is -NHR,where R is H, methyl or ethyl;

in Iamb: X is Orn or D-Orn,
A is H; and
C is NHR, where R is H, methyl or ethyl;

in Iamc: X is Lys;
A is H or acetyl; and
C is Gly or Gly-NHR, where R is H, methyl or ethyl;

in Iamd: X is Lys;
A is Ala; and
C is Gly or Gly-NHR, where R is H, methyl or ethyl;

in Iame: X is D-Lys;
A is H or acetyl; and
C is Gly or Gly-NHR, where R is H, methyl or ethyl;

in Iamf: X is Orn;
A is H or acetyl; and
C is Gly or Gly-NHR, where R is H, methyl or ethyl;

in Iamg: X is D-Orn;
A is H or acetyl; and
C is Gly or Gly-NHR, where R is H, methyl or ethyl;

in Iamh: X is Lys;
A is H or acetyl; and
C is Ala or Ala-NHR, where R is H, methyl or ethyl;

in Iami: X is D-Lys;
A is H or acetyl; and
C is Ala or Ala-NHR, where R is H, methyl or ethyl;

in Iamj: X is Lys;
A is H or acetyl; and
C is D-Ala or D-Ala-NHR, where R is H, methyl or ethyl;

in Iamk: X is D-Lys;
A is H or acetyl; and
C is D-Ala or D-Ala-NHR, where R is H, methyl or ethyl;

in Iaml: X is Orn;
A is H or acetyl; and
C is Ala or Ala-NHR, where R is H, methyl or ethyl;

in Iamm: Y is D-Orn;
A is H or acetyl; and
C is Ala or Ala-NHR, where R is H, methyl or ethyl;

in Iamn: X is Orn;
A is H or acetyl; and
C is D-Ala or D-Ala-NHR, where R is H, methyl or ethyl;

in Iamo: X is D-Orn;
A is H or acetyl; and
C is D-Ala or D-NHR, where R is H, methyl or ethyl;

in Iamp: X is Lys;
A is H or acetyl; and
C is Ser or Ser-NHR, where R is H,methyl or ethyl;

in Iamq: X is D-Lys;
A is H or acetyl; and
C is Ser or Ser-NHR, where R is H, methyl or ethyl;

in Iamr: X is Lys;
A is H or acetyl; and
C is D-Ser or D-Ser-NHR, where R is H, methyl or ethyl;

in Iams: X is D-Lys;
A is H or acetyl; and
C is D-Ser or D-Ser-NHR, where R is H, methyl or ethyl;

in Iamt: X is Orn;
A is H or acetyl; and
C is Ser or Ser-NHR, where R is H, methyl or ethyl;

in Iamu: X is D-Orn;
A is H or acety; and
C is Ser or Ser-NHR, where R is H, methyl or ethyl;

in Iav: X is Orn;

A is H or acetyl; and

C is D-Ser or D-Ser-NHR, where R is H, methyl or ethyl;

in Iamw: X is D-Orn;

A is H or acetyl; and

C is D-Ser or D-Ser-NHR, where R is H, methyl or ethyl;

in Iamx: X is Lys or D-Lys;

A is Ala or D-Ala; and

C is Gly;

in Iamy: X is Lys or D-Lys;

A is Ala or D-Ala; and

C is Ala or D-Ala;

in Iamz: X is Lys or D-Lys;

A is Ala or D-Ala; and

C is Ser or D-Ser;

in Ian: X is a residue of an amino acid or amino acid derivative with a positively charged side chain;

A is Ala, Val, Tyr, Asp, Sar, Ser, Thr, D-Ala, D-Val, D-Phe, D-Tyr, D-Asp, D-Ser or D-Thr;

C is Cys, Sar, Thr, HomoSer, D-Ala, D-Ser, D-Cys, D-Thr, D-Pro, D-Gln, D-Phe, D-Tyr, D-HomoSer, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Iao: X is Lys, Orn, Arg, His, D-Lys, D-Orn, D-Arg, or D-His;

A is Ala, Val, Tyr, Asp, Sar, Ser, Thr, D-Ala, D-Val, D-Phe, D-Tyr, D-Asp, D-Ser or D-Thr;

C is Cys, Sar, Thr, HomoSer, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Iap: X is Lys or D-Lys;

A is R, RCO, Ala, Gly, Val, Phe, Tyr, Asp, Sar, Ser, Thr, D-Ala, D-Val, D-Phe, D-Tyr, D-Asp, D-Ser, or D-Thr;

C is NH_2, NHR, OR, Gly, Ala, Ser, Cys, Sar, Thr, Pro, Gln, Phe, Tyr, HomoSer, D-Ala, D-Ser, D-Cys, D-Thr, D-Pro, D-Gln, D-Phe, D-Tyr, D-HomoSer, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR or OR; and

R is a C_1-C_8 lower alkyl;

in Iaq: X is Lys or D-Lys;

A is acetyl, Ala or D-Ala; and

C is NH_2, NHR, Gly, Ala, Ser, D-Ala, D-Ser, Gly-NHR, Ala-NHR, Ser-NHR, D-Ala-NHR, or D-Ser-NHR, where R is a C_1-C_8 alkyl;

in Iar: X is Orn or D-Orn;

A is H, R, RCO, Ala, Gly, Val, Phe, Tyr, Asp, Sar, Ser, Thr, D-Ala, D-Val, D-Phe, D-Tyr, D-Asp, D-Ser or D-Thr;

C is NH_2, NHR, NR_2, OR, or a residue of an amino acid or amino acid amide or ester formed by substitution of an amino acid with a substituent of the form NH_2, NHR, NR_2, or OR;

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

provided that Orn-Ala and Orn-NH_2 are not included;

in Ias: X is Orn or D-Orn;

A is H, R, RCO, Ala, Gly, Val, Phe, Tyr, Asp, Sar, D-Ala, D-Val, D-Phe, D-Tyr or D-Asp; and

C is NH_2, NHR, Gly, Ala, Ser, Cys, Sar, Thr, Pro, Gln, Phe, Tyr, HomoSer, D-Ala, D-Ser, D-Cys, D-Thr, D-Pro, D-Gln, D-Phe, D-Tyr, D-HomoSer, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR or OR;

R is a C_1-C_8 alkyl;

provided that Orn-Ala and Orn-NH_2 are not included;

in Iat: X is Orn or D-Orn;

A is H, acetyl, Ala or D-Ala; and

C is NH_2, NHR, Gly, Ala, Ser, D-Ala, D-Ser, Gly-NHR, Ala-NHR, Ser-NHR, D-Ala-NHR or D-Ser-NHR, wehre R is a C_1-C_8 alkyl;

provided that Orn-Ala and Orn-NH_2 are not included.

Specifically preferred are, furthermore, peptides of formulae Iba to Ibz and Ibza to Ibzo which correspond to formula Ib but wherein

in Iba: X and Y are residues of amino acids or amino acid derivatives with positively charged side chains;

A is H, R, RCO, amino acids, and amino acid derivatives formed by N -substitution of an amino acid with a substituent of the form R or RCO;

B is a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

C is OH, NH_2, NHR, NR_2, OR, or a residue of an amino acid or amino acid amide or ester formed by substitution of an amino acid with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibb: X and Y are residues of amino acids or amino acid derivatives with positively charged side chains;

A is H, R, RCO, or a residue of any amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

B is a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

C is OH, NH_2, NHR, NR_2, OR, or a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibc: X and Y are each residues of amino acids or amino acid derivatives with positively charged side chains;

A is H, R, RCO, Asp, Gly, Ala, Val, Leu, Asn, Glu, Ser, Thr, D-Asp, D-Ala, D-Val, D-Leu, D-Asn, D-Glu, D-Ser, D-Thr, or a residue of a derivative of one of the foregoing amino acids formed by N^{α}– substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Pro, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp, or a residue of a D-enantiomer of one of the foregoing amino acids;

C is OH, NH_2, NHR, NR_2, OR, Gly, Ser, Ala, Val, Leu, D-Ser, D-Ala, D-Val, D-Leu, or a residue of an amino acid amide or ester formed by substitution of one

of the foregoing amino acids with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibd: X and Y are each residues of amino acids or amino acid derivatives with positively charged side chains;

A is H, R, RCO, Asp, Gly, Ala, Val, Leu, Asn, Glu, Ser, Thr, D-Asp or a residue of a derivative of one of the foregoing amino acids formed by N^{α}- substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Pro, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp, D-Val, D-Ser, or D-Ala;

C is OH, NH_2, NHR, Gly, or Ser; and

R is a C_1- C_8 alkyl;

in Ibe: X and Y are each Lys, Arg, His, Orn, D-Lys, D-Arg, D-His, or D-Orn;

A is a chemical substituent, residue of an amino acid or amino acid derivative, or sequence of amino acids or amino acid derivatives, that preserves the immunoregulatory activity of said peptide;

B is a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide; and

C is a chemical substituent, residue of an amino acid or amino acid derivative, or sequence of amino

acids or amino acid derivatives, that preserves the immunoregulatory activity of said peptide;

in Ibf: X an Y are each Lys, Arg, His, Orn, D-Lys, D-Arg, D-His, or D-Orn;

A is H, R, RCO, or a residue of an amino acid or amino acid derivative formed by N^{α}-substitution of an amino acid with a substituent of the form R or RCO;

B is a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

C is OH, NH_2, NHR, NR_2, OR,or a residue of an amino acid or amino acid amide or ester formed by substitution of an amino acid with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibg: X and Y are each Lys, Arg, His, Orn, D-Lys, D-Arg, D-His, or D-Orn;

A is H, R, RCO, or a residue of amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

B is a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

C is OH, NH_2, NHR, NR_2, OR, or a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibh: X and Y are each Lys, Arg, His, Orn, D-Lys, D-Arg, D-His, or D-Orn;

A is H, R, RCO, Asp, Gly, Ala, Val, Leu, Asn, Glu, Ser, Thr, D-Asp, D-Ala, D-Val, D-Leu, D-Asn, D-Glu, D-Ser, D-Thr, or a residue of a derivative of one of the foregoing amino acids formed by N^{α}-substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Pro, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp, or a residue of one of the D-enantiomers of the foregoing amino acids;

C is OH, NH_2, NHR, NR_2, OR, Gly, Ser, Ala, Val, Leu, D-Ser, D-Ala, D-Val, D-Leu, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibi: X and Y are each Lys,Arg, His, Orn, D-Lys, D-Arg, D-His, or D-Orn;

A is H, R, RCO, Asp, Gly, Ala, Val,Leu, Asn, Glu, Ser, Thr, D-Asp or a residue of a derivative of one of the foregoing amino acids formed by N -substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Pro, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp, D-Val, D-Ser, or D-Ala;

C is OH, NH_2, NHR, Gly or Ser; and

R is a C_1-C_8 alkyl;

in Ibj: X and Y are each Lys, Arg, His, or Orn;

A is a chemical substituent, a residue of an amino acid or amino acid derivative, or sequence of amino acids or amino acid derivatives, that preserves the immunoregulatory activity of said peptide;

B is a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide; and

C is a chemical substituent, a residue of an amino acid or amino acid derivative, or sequence of amino acids or amino acid derivatives, that preserves the immunoregulatory activity of said peptide;

in Ibk: X and Y are each Lys, Arg, His, or Orn;

A is H, R, RCO, or a residue of an amino acid or amino acid derivative formed by N^{α}-substitution of an amino acid with a substituent of the form R or RCO;

B is a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

C is OH, NH_2, NHR, NR_2, OR, or a residue of an amino acid or amino acid amide or ester formed by substitution of

an amino acid with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibl: X and Y are each Lys, Arg, His, or Orn;

A is hydrogen, R, RCO, or a residue of amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

B is a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide;

C is OH, NH_2, NHR, NR_2, OR, or a residue of an amino acid or amino acid derivative that preserves the immunoregulatory activity of said peptide; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibm: X and Y are each Lys, Arg, His, or Orn;

A is H, R, RCO, Asp, Gly, Ala, Val, Leu, Asn, Glu, Ser, Thr, D-Asp, D-Ala, D-Val, D-Leu, D-Asn, D-Glu, D-Ser, D-Thr, or a residue of a derivative of one of the foregoing amino acids formed by N^{α}-substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Pro, Asn, Asp, Arg, His, Ile, Thr, Phe, Lys, Orn, Trp, or a residue of a D-enantiomer of one of the foregoing amino acids;

C is OH, NH_2, NHR, NR_2, OR, Gly, Ser, Ala, Val, Leu, D-Gly, D-Ser, D-Ala, D-Leu, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or C_3-C_{14} cycloalkyl;

in Ibn: X and Y are each Lys, Arg, His, or Orn;

A is H, R, RCO, Asp, Gly, Ala, Val, Leu, Asn, Glu, Ser, Thr, D-Asp or a residue of a derivative of one of the foregoing amino acids formed by N -substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Pro, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp, D-Val, D-Ser, or D-Ala;

C is OH, NH_2, NHR, Gly or Ser; and

R is a C_1-C_8 alkyl;

in Ibo: X is Lys, Arg, His, or Orn;

Y is Lys, Arg, or His;

A is Ac, Gly, Ala, Val, Asp, Asn, Glu, Arg, Ser, Thr, D-Asp, Ac-Asn, Val-Asp-, Gly-Asp-, Ala-Asp-, Ser-Asp-, Thr-Val-Asp-, or Leu-Thr-Val-Asp-;

B is Val, Ser, Thr, Gly, Ala, Asp, Lys, or D-Ser; and

C is OH, or NHR, where R is H or C_1-C_8 alkyl;

in Ibp: X and Y are each Lys;

A is H;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Pro, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, D-Ala, or D-Ser; and

C is OH.

in Ibq: X and Y are each Lys;

A is H;

B is Val, Leu, Glu, Gln, Ser, Gly, Pro, Tyr, Phe, Lys, Trp, or D-Val; and

C is NHR, where R is H or a C_1-C_8 alkyl;

in Ibr: X is Lys;

Y is Arg;

A is H,

B is Ser, Thr, Gly, or Ala; and

C is OH or NHR, where R is hydrogen or a C_1-C_8 alkyl;

in Ibs: X is Arg;

Y is Lys;

A is H;

B is Val, Ser, Thr, Gly, or Ala; and

C is OH or NHR, where R is H or a C_1-C_8 alkyl;

in Ibt: X is Arg;

Y is Lys;

A is H;

B is Asp or Ser;

C is OH or NHR, where R is H or a C_1-C_8 alkyl;

in Ibu: X is Lys;

Y is Lys or Arg;

A is Gly, Ala, Ser, Thr, Val, or Ac-Asn;

B is Val; and

C is OH or NHR, where R is H or a C_1-C_8 alkyl;

in Ibv: X is Lys,

y is Lys or Arg;

A is Ala, Val, Ser, Thr, or Ac-Asn;

B is Thr; and

C is OH or NHR, where R is H or a C_1C_8 alkyl;

in Ibw: X is Lys;

Y is Lys or Arg;

A is Ac-, Ala, Asn, Glu, Gly, Arg, Ac-Asn, Gly-Asp-, Ala-Asp-, Val-Asp-, Ser-Asp-, or Thr-Val-Asp-;

B is Ser; and

C is OH or NHR, where R is H or a C_1-C_8 alkyl;

in Ibx: X and Y are residues of amino acids or amino acid derivatives with positively charged side chains;

A is H, R, RCO, a residue of an amino acid or amino acid derivative formed by N^{α}-substitution of an amino acid with a substituent of the form R or RCO, provided that A is not Thr if X is Lys and Y is Arg or a derivative of Arg;

B is Val, Leu, Gln, Ser, Thr, Gly, Ala, Asp, Arg, His, Ile, Tyr, Phe, Lys, Sar, D-Thr, or D-Pro;

C is OH, NH_2, NHR, NR_2, OR, Gly, Ser, Ala, Val, Leu, D-Ser, D-Ala, D-Val, D-Leu, or a residue of an

amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or a C_3-C_{14} cycloalkyl;

in Iby: X and Y are residues of amino acids or amino acid derivatives with positively charged side chains:

A is H, R, RCO, a residue of an amino acid or amino acid derivative formed by N^{α}-substitution of an amino acid with a substituent of the form R or RCO;

B is Glu, Asn, Orn, Trp, D-Val, D-Ser, D-Ala, D-Leu, D-Glu, D-Gln, D-Ala, D-Asn, D-Asp, D-Arg, D-His, D-Ile, D-Tyr, D-Phe, D-Lys, D-Orn, D-Trp, or a residue of a derivative of one of the foregoing amino acids;

C is OH, NH_2, NHR, NR_2, OR,Gly, Ser, Ala, Val, Leu, D-Ser, D-Ala, D-Val, D-Leu, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or a C_3-C_{14} cycloalkyl;

in Ibz: X and Y are residues of amino acids or amino acid derivatives with positively charged side chains;

A is H, R, RCO, Asp, Gly, Ala, Val, Leu, Asn, Glu, Ser, D-Asp or a residue of a derivative of one of the foregoing amino acids formed by N^{α}-substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp, D-Val, D-Ser, or D-Ala;

C is OH, NH_2, NHR, Gly or Ser; and

R is a C_1-C_8 alkyl;

in I bza: X and Y are each Lys, Arg, His, Orn, D-Lys, D-Arg, D-His or D-Orn;

A is H, R, RCO, a residue of an amino acid or amino acid derivative formed by N^{α}-substitution of an amino acid with a substituent of the form R or RCO, provided that A is not Thr if X is Lys and Y is Arg or a derivative of Arg;

B is Val, Leu, Gln, Ser, Thr, Gly, Ala, Asp, Arg, His, Ile, Tyr, Phe, Lys, Sar, D-Thr, or D-Pro;

C is OH, NH_2, NHR, NR_2, OR, Gly, Ser, Ala, Val, Leu, D-Ser, D-Ala, D-Val, D-Leu, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or a C_3-C_{14} cycloalkyl;

in Ibzb:X and Y are each Lys, Arg, His, Orn, D-Lys, D-Arg, D-His or D-Orn;

A is H, R, RCO, a residue of an amino acid or amino acid derivative formed by N^{α}-substitution of an amino acid with a substituent of the form R or RCO;

B is Glu, Asn, Orn, Trp, D-Val, D-Ser, D-Ala, D-Leu, D-Glu, D-Gln, D-Ala, D-Asn, D-Asp, D-Arg, D-His, D-Ile, D-Tyr, D-Phe, D-Lys, D-Orn, D-Trp, or a residue of a derivative of one of the foregoing amino acids;

C is OH, NH_2, NHR, NR_2, OR, Gly, Ser, Ala, Val, Leu, D-Ser, D-Ala, D-Val, D-Leu, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1- C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or a C_3-C_{14} cycloalkyl;

in Ibzc: X and Y are each Lys, Arg, His, Orn, D-Lys, D-Arg, D-His, or D-Orn;

A is H, R, RCO, Asp, Gly, Ala, Val, Leu, Asn, Glu, Ser, D-Asp or a residue of a derivative of one of the foregoing amino acids formed by N -substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp, D-Val, D-Ser, or D-Ala;

C is OH, NH_2, NHR, Gly or Ser; and

R is a C_1-C_8 alkyl;

in Ibzd: X and Y are each Lys, Arg, His, or Orn;

A is H, R, RCO, a residue of an amino acid or amino acid derivative formed by N^{α}-substitution of an amino acid with a substituent of the form R or RCO, provided that A is not Thr if X is Lys and Y is Arg or a derivative of Arg;

B is Val, Leu, Gln, Ser, Thr, Gly, Ala, Asp, Arg, His, Ile, Tyr, Phe, Lys, Sar, D-Thr, or D-Pro;

C is OH, NH_2, NHR, NR_2, OR, Gly, Ser, Ala, Val, Leu, D-Ser, D-Ala, D-Val, D-Leu, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or a C_3-C_{14} cycloalkyl;

in Ibze: X and Y are each Lys, Arg, His, or Orn;

A is H, R, RCO, a residue of an amino acid or amino acid derivative formed by N^{α}-substitution of an amino acid with a substituent of the form R or RCO;

B is Glu, Asn, Orn, Trp, D-Val, D-Ser, D-Ala, D-Leu, D-Glu, D-Gln, D-Ala, D-Asn, D-Asp, D-Arg, D-His, D-Ile, D-Tyr, D-Phe, D-Lys, D-Orn, D-Trp, or a residue of a derivative of one of the foregoing amino acids;

C is OH, NH_2, NHR, NR_2, OR, Gly, Ser, Ala, Val, Leu, D-Ser, D-Ala, D-Val, D-Leu, or a residue of an amino acid amide or ester formed by substitution of one of the foregoing amino acids with a substituent of the form NH_2, NHR, NR_2, or OR; and

R is a C_1-C_8 aliphatic, C_6-C_{14} aryl, aralkyl or alkaryl, or a C_3-C_{14} cycloalkyl;

in Ibzf: X and Y are each Lys, Arg, His, or Orn;

A is H, R, RCO, Asp, Gly, Ala, Val, Leu, Asn, Glu, Ser, D-Asp or a residue of a derivative of one of the foregoing amino acids formed by N -substitution with a substituent of the form R or RCO;

B is Val, Leu, Glu, Gln, Ser, Thr, Gly, Ala, Asn, Asp, Arg, His, Ile, Tyr, Phe, Lys, Orn, Trp, D-Val, D-Ser, or D-Ala;

C is OH, NH_2, NHR, Gly or Ser; and

R is a C_2-C_8 alkyl;

in Ibzg: X is Lys, Arg, His, or Orn;

Y is Lys, Arg, or His;

A is Ac, Gly, Ala, Val, Asp, Asn, Glu, Arg, Ser, Thr, D-Asp, Ac-Asn, Val-Asp-, Gly-Asp-, Ala-Asp-, Ser-Asp-, Thr-Val-Asp-, or Leu-Thr-Val-Asp-;

B is Val, Ser, Thr, Gly, Ala, Asp, Lys, or D-Ser; and

C is OH, or NHR, where R is H or C_1-C_3 alkyl;

in Ibzh: X and Y are each Lys;

A is H;

B is Val, Leu, Glu, Gln, Ser, Thr, Ala, Asn, Arp, Arg, His, Ile, Tyr, Phe, Lys, Orn, D-Ala, or D-Ser; and

C is OH;

in Ibzi: X and Y are each Lys;

A is H;

B is Val, Leu, Glu, Gln, Ser, Gly, Pro, Tyr, Phe, Lys, Trp, or D-Val; and

C is NHR, where R is H or a C_1-C_3 alkyl;

in Ibzj: X is Lys;

Y is Arg;

A is H;

B is Ser, Thr, Gly, or Ala; and

C is OH or NHR, where R is H or a C_1-C_3 alkyl;

in Ibzk: X is Arg;

Y is Lys;

A is H;

B is Val, Ser, Thr, Gly or Ala; and

C is OH or NHR, where R is hydrogen or a C_1-C_3 lower alkyl;

in Ibzl: X is Arg;

Y is Lys;

B is Asp or Ser;

C is OH or NHR, where R is hydrogen or a C_1-C_3 alkyl;

in Ibzm: X is Lys;

Y is Lys or Arg;

A is Gly, Ala, Ser, Val, or Ac-Asn;

B is Val; and

C is OH or NHR, where R is hydrogen or a C_1-C_3 alkyl;

in Ibzn: X is Lys;

Y is Lys or Arg;

A is Ala, Val, Ser, or Ac-Asn;

B is Thr; and

C is OH or NHR, where R is H or a C_1-C_3 alkyl;

in Ibzo: X is Lys;

Y is Lys or Arg;

A is Ac, Ala, Asn, Glu, Gly, Arg, Ac-Asn, Gly-Asp-, Ala-Asp-, Val-Asp-, Ser-Asp-, or Thr-Val-Asp-;

B is Ser; and

C is OH or NHR, where R is H or a C_2-C_3 alkyl.

The peptides of formula I can be prepared by usual methods of peptide synthesis.

In particular, a peptide of formula I is prepared by liberating it from its corresponding functional derivative which may be bound by a covalent bond to a solid resin by treatment under acidic or basic conditions, and, if desired, the peptide thus obtained is esterified, amidated and/or acylated to yield a corresponding ester, N- and/or O-acyl derivative or is transformed into one of its pharmacologically acceptable salts by treatment with a base or an acid.

Immunoregulatory Activity of the Subject Peptides

As discussed above, T-lymphocytes (T cells) play a powerful role in the *in vivo* induction, suppression, and regulation of immune responses leading to a number of autoimmune and other disease conditions. In order to examine the effect of peptides or other drugs on T cell function, *in vitro* assays have been developed which are predictive of *in vivo* T cell function. In the usual *in vitro* assays of T cell function, lymphoid cells from peripheral blood or from the spleen, lymph nodes or other organs are isolated and cultured with or without potential immunoregulatory drugs. The extent to which lymphocyte function in general and T cell function in particular are regulated may be assessed by many measurement techniques. The most common technique measures the amount of a radioactive precursor of DNA (tritiated thymidine) incorporated into newly synthesized DNA of lymphocytes. Since only "activated" cells that are preparing to divide synthesize new DNA, the amount of tritiated thymidine incorporated in cells and the resultant incorporated radioactivity is directly related to the extent of cell activation and resultant proliferation.

In order to predict whether a potential immunoregulatory drug will be useful in treating a particular disease or class of diseases, it is important that it be tested in *in vitro* or *in vivo* system that are reflective of the particular disease or diseases in question. Several *in vitro* assays of T cell function in particular are highly predictive of *in vivo* efficacy in treating organ transplantation rejection and autoimmune disease.

The mixed lymphocyte response (MLR) is an *in vitro* analog of the *in vivo* organ transplantation rejection response. To perform the MLR, T cell-containing lymphocyte preparations from two humans or animals are cultured together for five to seven days under standard cell culture conditions. During the culture, T cells from both subjects recognize histocompatibility molecules of the other subject. If both subjects are genetically identical, their histocompatiblity molecules will also be identical and no activation or cell proliferation will occur. The radioactivity incorporated into cells will therefore be very low since few cells are stimulated to divide. By contrast, if both subjects are genetically non-identical, both subjects' cells will recognize the foreign histocompatibility molecules of the other and will divide and proliferate. The level of cellular radioactivity will therefore be increased over that of control, unstimulated cultures and will be directly proportional to the magnitude of the genetic difference between the two subjects. This assay is termed a two-way MLR since each subject's cells respond to the other's proliferative stimuli. If one of the subject's cells is prevented from dividing by, for example, exposure to gamma ir-

radiation or mitomycin C, then the observed proliferation and incorporated radioactivity will be due only to the other subject's cellular proliferation. This assay is termed a one-way MLR.

During a MLR, some of the proliferating T cells differentiate into T cells able to kill the target cells which produced the initial stimulus. These sensitized cytotoxic or Killer T cells are primarily directed toward the target cell's histocompatibility molecules and produce T cell-mediated destruction which closely resembles the destruction which occurs during organ transplantation rejection. If T cells from a transplant recipient are sensitized to the histocompatibility antigens of a transplanted organ in vitro and then injected into the recipient, the sensitized T cells will cause the organ to be rejected. Sensitized T cells alone are therefore both necessary and sufficient to cause organ transplant rejection. As demonstrated in Table 1, the peptides of the present invention can substantially suppress the MLR without significantly affecting cell viability. This indicates that such peptides have therapeutic usefulness since they can suppress the immune response responsible for the immunologic rejection of transplanted organs and autoimmune diseases.

It should be noted that suppression of T cell activities in vitro by peptides of the present invention is but one manifestation of the complex immunoregulation which occurs in vitro and in vivo and which leads to reduction or prevention of autoimmune disease or of the rejection of transplanted organs. For example, while the peptides of the present invention can suppress a MLR at

concentrations listed in table 1, lower peptide concentrations typically result in stimulation of tritiated thymidine uptake which is reflective of the peptides' ability to activate certain immunoregulatory processes.

Administration of the subject peptides to animals, for example, can in certain cases result in stimulation of certain T lymphocyte subsets, which can in turn lead to net suppression of a clinical disease or condition. It is thus entirely within the scope of the peptides of the present invention that certain immunostimulatory properties, in addition to the suppressive properties described herein, will be expressed in alternate *in vitro* and *in vivo* tests of the subject peptides.

The MLR is a specific example of a more general T cell response toward "foreign" antigens. In a MLR, T cells recognize and proliferate in response primarily to antigens located on histocompatibility molecules of the stimulator cells. Molecules unrelated to histocompatibility antigens may also serve as potent stimulators of T cell proliferative and killing responses. In fact virtually any molecule, if presented to T cells in an appropriate manner, may stimulate T cell proliferative, regulatory and killing responses directed toward the stimulating antigen. Such antigen-specific T cell responses in humans are thought to be responsible for many of the autoimmune diseases listed earlier when immune tolerance is reduced or lost.

Specific *in vitro* assays exist which can measure a T cell response directed toward any specific antigen. In

particular, the therapeutic and immunoregulatory utility of the peptides of the present invention is further demonstrated by an antigen-specific T cell proliferation assay which uses mice that have been immunized with a particular antigen such as Bovine Serum Albumin (BSA). Upon immunization, T cells in lymph nodes which drain the injection site are activated and stimulated to proliferate. During this immune response, regulatory and Killer T cells which specifically recognize BSA are generated. At six days post-immunization when the T cell response is sufficiently mature, the draining lymph nodes are removed and cells eluted from them are placed into a standard culture. BSA or a second, unrelated antigen is then added to the cultures with or without the immunoregulatory peptides of the present invention. Quantitation of antigen-induced T cell activation and proliferation is assessed by cellular tritiated thymidine incorporation at the end of the culture incubation in a manner similar to that used in MLR cultures. Cultures containing BSA will produce high levels of tritiated thymidine incorporation because the BSA-sensitized T cells recognize the BSA molecules and become stimulated to proliferate. Cultures containing an unrelated antigen, by contrast, have low stimulation levels because they lack receptors directed toward the unrelated antigen.

As demonstrated in Table 2, the peptides of the present invention substantially suppress the T cell proliferative response toward specific molecular or cellular antigens without significantly affecting cellular viability. This observation is

of therapeutic importance because it indicates that such peptides can suppress an abnormal T cell immune response directed toward a wide range of unrelated antigens different from those found on histocompatibility antigens. As previously discussed, abnormal Killer T cell responses directed against either foreign or normal "self" antigens on various organs are thought to be responsible for many autoimmune diseases. Other autoimmune diseases may result from an abnormal regulatory T cell response towards specific antigens that result in an imbalanced response of other T cell-regulated portions of the immune system.

It is possible to develop <u>in vivo</u> models of human autoimmune disease in which animals are immunized with organ tissues from healthy genetically identical animals. Animals immunized against one of their organs develop an autoimmune response which may result in destruction of the organ. T cells isolated from such an immunized animal may be very specific for molecules and cells of the injected organ showing that the T cells have receptors for organ-specific antigens.

By using organs or their preparations as immunizing agents as described, it is possible to develop animal models of autoimmune diseases involving virtually any organ system, some of which were previously enumerated.

The peptides of the present invention are efficacious in reducing or preventing the tissue destruction characteristic of autoimmune disease <u>in vivo</u>. Figures 1 and 2 demonstrate the efficacy of one peptide and one of its peptidomimetic analogs in

treating an animal model of human multiple sclerosis (MS). In this model, termed Experimental Allergic Encephalomyelitis (EAE), SJL/J mice were immunized with a homogenate of spinal cord from SJL/J mice in a Complete Freund's Adjuvant medium. This homogenate contains cells and molecules representative of the many potential antigens which are present in the central nervous system (CNS). Such animals typically develop vestibular and occular disturbances, weakness, paralysis, and other signs of central nervous system destruction characteristic of both EAE and MS ten to fifteen days after initial immunization. The EAE model is discussed in Brown et al., Lab. Invest. 45(3):278-284 (1981).

Like the majority of human MS cases, the disease severity in this animal MS model waxes and wanes during the disease course. Mice receiving only saline injections two to three times per week exhibit high peaks and troughs of disease severity. By contrast, mice receiving peptide or peptidomimetic demonstrated very mild disease initially and were clinically disease free by the study's end. Microscopic analysis of brain sections from both peptide and saline-treated mice is presented in Table 3. One hundred percent of saline-treated mice demonstrated substantial numbers of visible lesions which represent physical brain destruction in all portions of the CNS examined. By contrast, sixty percent of peptide-treated mice were lesion-free while the remaining forty percent of peptide-treated mice had few, scattered lesions confirming the clinically-elicited observations.

In addition to the immunoregulatory properties of the peptides of the present invention, the peptides may also be used to regulate the growth of neoplastic (cancer) cells in vivo. All cancer cells have in common the property of unregulated cell replication. Whereas normal cells replicate at a rate consistent with the normal functioning of the organ in which the cells reside, cancerous cells replicate in an unregulated manner, which leads to tumor formation and possible destruction of adjacent healthy tissue.

As demonstrated in Tables 4 and 5, the peptides of the present invention can suppress or prevent the growth of a variety of neoplastic cells _in vitro_. Figure 3 demonstrates the anti-cancer properties of one of the peptides of the present invention in mice with lymphoma/leukemia of T cell origin. Whereas saline treated mice had a median survival of 23.5 days after injection of lymphoma/leukemia cells, peptide-treated mice had a median survival of 30.5 days. Additionally, all saline-treated mice were dead by day 31 while one peptide-treated mouse was tumor-free at day 50.

The peptides of the present invention may be administered to humans or other mammals by a variety of means commonly utilized with respect to other therapeutic agents. Injected subcutaneously, a dosage range of from about 25μg/kg to about 50 mg/kg of body weight may be employed, with a preferred range of about 2.5 to 50 mg/kg. Sterile saline, aqueous dextrose and

glycols are preferred liquid carriers, particularly (when isotonic) for injectable solutions. Intravenous, intraperitoneal, and intramuscular injections may also be used at dosages comparable to those of subcutaneous injections. The peptides may be administered orally at dosages generally ranging from one to 100 times that of an injected dose, although greater dosages may be required depending on factors such as food intake. The peptides may be combined with appropriate pharmaceutical carriers in the form of pills, capsules, elixers, suspensions, aerosols, powers, or in other forms. Intranasal administration may be achieved by using an appropriate amphipathic vehicle such as, for example, glycocholic acid or EDTA. Administration may be achieved topically or transdermally, using an appropriate carrier in the form of, for example, an ointment. Sublingual, rectal, or topical ocular administration using appropriate pharmaceutical media may be employed. Sustained-release formulations using, for example, liposome delivery media, are also appropriate vehicles for administering the subject peptides.

TABLE 1

PERCENT INHIBITION OF DNA PROLIFERATION OF MURINE AND HUMAN LYMPHOCYTES DURING MIXED LYMPHOCYTE CULTURES IN THE PRESENCE OF THE SUBJECT PEPTIDES

Peptide Sequence	Murine MLR [Peptide] (ug/ml)	Murine MLR % Inhib[1/]	Human MLR [Peptide] (ug/ml)	Human MLR % Inhib[1/]
Lys-NH_2	100	98	200	99
D-Lys-NH_2	50	99	200	99
D-Lys-$NH_2CH_2CH_3$	200	99	400	86
Lys-Ser	50	99	200	99.
D-Lys-Ser	50	99	400	14
Lys-Ser-NH_2	50	99	100	99
desamino-Lys-Ser	200	98	400	99
Ac-Lys-Ser	50	99	400	71
Ac-Lys-Ser-NH_2	400	92	400	44
N-CH_3-Lys-D-Ser	200	96	400	97
Orn-Ala	50	99	100	91
Orn-D-Ala	50	98	100	94
Orn-Gly	100	99	200	97
Arg-D-Ala	200	99	400	88
Arg-Sar	200	98	400	97
Arg-Thr	400	83	50	-32
His-Ser	100	99	400	97
Lys-Cys	200	99	100	93
Lys-Phe	100	99	400	99
Lys-D-Phe	400	-61	400	54
Lys-Tyr	200	99	400	95
Lys-Asp	100	99	100	-41
Lys-Gln	100	99	400	55
Lys-Leu	100	99	200	26
Lys-Pro	200	99	200	-19
Lys-HomoSer	200	99	200	93
Ala-Lys-Ser	50	99	50	90
Ala-Lys-Gly	50	99	200	90
D-Ala-D-Lys-D-Ser	50	96	200	90
Gly-Lys-Ser	200	89	400	94
Val-Lys-Ser	100	-59	400	99
Phe-Lys-D-Ala	50	99	--	--
Phe-Lys-Sar	400	99	100	-100
Tyr-Lys-Gly	200	99	400	94
Asp-Lys-Ser	50	99	400	99

[Table 1 continues next page]

[Table 1, continued]

Peptide Sequence	Murine MLR [Peptide] (ug/ml)	Murine MLR % Inhib[1]/	Human MLR [Peptide] (ug/ml)	Human MLR % Inhib[1]/
Sar-D-Lys-Ser	100	99	400	58
Lys-Ser-Tyr	200	95	400	99
Lys-Ser-Ala	200	98	400	99
Phe-Lys-Ser-Tyr	200	98	400	96
Lys-Val-Lys	400	99	100	96
Lys-Glu-Lys	50	99	400	84
Lys-Gln-Lys	100	98	100	85
Lys-Arg-Lys	100	99	100	76
Lys-His-Lys	100	99	100	77
Lys-D-Ala-Lys	200	99	200	43
Lys-Val-Lys-NH_2	100	99	100	96
Lys-Leu-Lys-NH_2	50	99	100	89
Lys-Pro-Lys-NH_2	50	99	100	92
Lys-Tyr-Lys-NH_2	100	99	400	98
Lys-D-Val-Lys-NH_2	400	99	400	63
Lys-Ser-Arg	100	92	100	49
Lys-Thr-Arg	200	98	200	-47
Arg-Gly-Lys	200	98	400	-31
Arg-Ala-Lys	100	92	50	-39
Arg-Asp-Arg	50	99	200	92
Ac-Lys-Ser-Lys	400	97	400	99
Val-Lys-Val-Lys-NH_2	100	99	200	99
Ala-Lys-Val-Lys-NH_2	50	98	100	90
Ala-Lys-Ser-Arg	400	99	400	-132
Arg-Lys-Ser-Arg	100	96	100	-141
Asn-Lys-Ser-Arg	50	70	400	99
Ac-Asn-Lys-Ser-Arg	200	99	400	99
Gly-Asp-Lys-Ser-Arg	100	90	400	51
Asp-Lys-Lys-Arg	100	99	100	86
Asp-Lys-Ile-Arg	200	99	400	42
Ser-Lys-Val-Arg	100	98	400	85
Gly-Lys-Val-Arg	100	99	100	60
Asp-Orn-Ser-Arg	100	99	200	99
Asp-His-Ser-Arg	400	95	400	-36
Asp-Lys-Ser-His	100	90	400	26

/Table 1 continues next page/

/Table 1, continued/

1/ Percentage inhibition as compared to cultures in the absence of peptide.

NOTE: Splenocytes from Balb/c mice were cocultured with splenocytes from C57B/6 mice and one healthy human donor were cultured with irradiated stimulator cells from a second healthy human donor for seven days at 37°C and 5% CO_2. Cultures were pulsed with 1 μCi ^3H-thymidine for six hours.

The concentrations of the subject peptides which produce inhibition or stimulation of proliferation may occasionally vary from the concentrations listed depending on many factors including age of the donor, time of day of blood drawing, food intake and other factors.

TABLE 2

INHIBITION OF ANTIGEN-SPECIFIC DNA PROLIFERATION OF SENSITIZED MURINE LYMPHOCYTES IN THE PRESENCE OF THE SUBJECT PEPTIDES

Peptide Sequence	Concentration (µg/ml)	% Inhibition[1/]
Lys-NH_2	100	98
D-Lys-NH_2	100	99
D-Lys-$NH_2CH_2CH_3$	100	36
Lys-Ser	50	99
D-Lys-Ser	12.5	96
Lys-Ser-NH_2	25	92
desamino-Lys-Ser	100	68
Ac-Lys-Ser	50	98
Ac-Lys-Ser-NH_2	50	99
N-Ch_3-Lys-D-Ser	100	99
Orn-D-Ala	50	99
Orn-Gly	12.5	94
Arg-D-Ala	12.5	36
Arg-Sar	100	99
Arg-Thr	25	22
His-Ser	100	-13
Lys-Cys	100	74
Lys-Phe	50	99
Lys-Tyr	100	75
Lys-Asp	100	92
Lys-Pro	100	44
Ala-Lys-Ser	50	98
Ala-Lys-Gly	100	99
D-Ala-D-Lys-D-Ser	100	-31
Phe-Lys-D-Ala	100	99
Asp-Lys-Ser	100	99
Sar-D-Lys-Ser	100	18
Lys-Ser-Tyr	100	60
Lys-Ser-Ala	12.5	-57

/Table 2 continues next page/

[Table 2 continued]

Peptide Sequence	Concentration (µg/ml)	%Inhibition[1/]
Lys-Val-Lys	50	-96
Lys-Glu-Lys	12.5	98
Lys-Arg-Lys	50	100
Lys-His-Lys	100	99
Lys-D-Ala-Lys	100	62
Lys-Val-Lys-NH_2	50	97
Lys-Leu-Lys-NH_2	50	94
Lys-Thr-Arg	25	-7
Arg-Gly-Lys	50	11
Arg-Ala-Lys	100	7
Arg-Asp-Arg	25	99
Val-Lys-Val-Lys-NH_2	50	99
Ala-Lys-Val-Lys-NH_2	50	99
Asn-Lys-Ser-Arg	100	99
Asp-Orn-Ser-Arg	25	94

1/ Percentage inhibition as compared to cultures in the absence of peptide.

NOTE: Balb/c mice were immunized with ovalbumin (OVA) in Complete Freund's Adjuvant. Seven days later lymphocytes from superficial inguinal lymph nodes were cultured with 100 µg/ml OVA and peptide. Cultures were pulsed with 1 µCi ^{3}H-thymidine for 24 hours from day 4 to day 5.

The concentrations of the subject peptides which produce inhibition or stimulation of proliferation may occasionally vary from the concentrations listed depending on many factors including age of the donor, time of day of blood drawing, food intake and other factors.

TABLE 3

PATHOLOGICAL LESIONS PRESENT IN CENTRAL NERVOUS TISSUE OF MICE DURING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS TREATED WITH SALINE OR SUBJECT PEPTIDE LYS-SER

CNS TISSUE	SUBCUTANEOUS INJECTIONS* SALINE (8)	SUBCUTANEOUS INJECTIONS* LYS-SER (10)
Spinal Cord	8.1 ± 3.9	1.2 ± 1.0 (85)
Cerebellum	20.1 ± 6.7	0.6 ± 0.4 (97)
Brainstem	12.3 ± 6.5	0.4 ± 0.2 (97)
Basal Nuclei	2.6 ± 2.1	1.2 ± 0.9 (54)
Cerebral White Matter	2.8 ± 0.9	1.8 ± 1.2 (36)
Cerebral Grey Matter	4.6 ± 2.2	1.3 ± 0.9 (72)

*/ Mean ± standard error of lesions detected per mouse.

NOTE: SJL/J mice were injected subcutaneously with mouse spinal cord homogenate in Complete Freund's Adjuvant on days 0 and 7 and injected with saline or 1 mg peptide 3 times per week from day 11 to 32. The number of mice in each group is enclosed within parentheses. Tissue was sectioned, the sample identity was coded and was examined microscopically by an uninformed observer. The percent inhibition of lesions is given in the last column in parentheses.

TABLE 4
PERCENT INHIBITION OF DNA PROLIFERATION OF MURINE LEUKEMIC CELLS IN THE PRESENCE OF THE SUBJECT PEPTIDES

Peptide Sequence	L 1210 Cells [Peptide] (µg/ml)	% Inhib.[1]	WEHI-22 Cells [Peptide] (µg/ml)	% Inhib.[1]
Lys-NH_2	100	98	200	24
D-Lys-NH_2	200	98	200	72
Lys-Ser	200	78	200	37
D-Lys-Ser	200	-13	200	26
desamino-Lys-Ser	200	21	200	3
Ac-Lys-Ser	200	44	200	25
Ac-Lys-Ser-NH_2	50	-57	200	18
N-CH_3-Lys-D-Ser	200	37	200	74
Orn-Ala	100	71	100	81
Orn-D-Ala	100	89	100	86
Orn-Gly	100	95	100	86
Arg-D-Ala	200	89	200	80
Arg-Sar	200	97	100	77
His-Ser	200	67	200	71
Lys-Cys	50	94	100	99
Lys-Phe	200	99	--	--
Lys-D-Phe	200	19	200	-27
Lys-Asp	200	38	200	8
Lys-Gln	200	-34	200	7
Lys-Leu	200	69	200	40
Lys-Pro	100	8	100	24
Ala-Lys-Ser	100	49	200	95
Ala-Lys-Gly	200	83	200	54
D-Ala-D-Lys-D-Ser	100	29	100	40
Gly-Lys-Ser	200	91	200	91
Val-Lys-Ser	200	80	200	75
Phe-Lys-Sar	200	16	200	17
Tyr-Lys-Gly	50	-18	100	-18
Asp-Lys-Ser	--	--	100	39
Sar-D-Lys-Ser	200	81	200	72
Lys-Ser-Tyr	100	10	--	--
Lys-Ser-Ala	200	98	200	89
Phe-Lys-Ser-Tyr	50	-7	50	-35

[Table 4 continues next page]

/Table 4, continued/

Peptide Sequence	L 1210 Cells [Peptide] (µg/ml)	L 1210 Cells % Inhib.1/	WEHI-22 Cells [Peptide] (µg/ml)	WEHI-22 Cells % Inhib.1/
Lys-Val-Lys	100	64	200	85
Lys-Glu-Lys	200	43	200	43
Lys-Gln-Lys	200	99	200	94
Lys-Arg-Lys	200	73	100	50
Lys-His-Lys	200	99	200	94
Lys-D-Ala-Lys	25	-19	100	-83
Lys-Val-Lys-NH_2	200	93	100	82
Lys-Leu-Lys-NH_2	200	96	200	96
Lys-Pro-Lys-NH_2	100	95	100	90
Lys-Tyr-Lys-NH_2	200	85	200	81
Lys-D-Val-Lys-NH_2	200	28	100	24
Lys-Ser-Arg	100	28	100	19
Lys-Thr-Arg	200	17	200	10
Arg-Gly-Lys	200	19	25	30
Arg-Ala-Lys	100	-13	200	-22
Arg-Asp-Arg	100	88	100	94
Ac-Lys-Ser-Lys	--	--	200	58
Val-Lys-Val-Lys-NH_2	200	88	200	85
Ala-Lys-Val-Lys-NH_2	200	65	200	76
Ala-Lys-Ser-Arg	--	--	100	16
Arg-Lys-Ser-Arg	--	--	100	-31
Asn-Lys-Ser-Arg	--	--	100	16
Ac-Asn-Lys-Ser-Arg	--	--	100	-41
Gly-Asp-Lys-Ser-Arg	--	--	100	-14
Asp-Lys-Lys-Arg	--	--	100	81
Asp-Lys-Ile-Arg	--	--	100	21
Ser-Lys-Val-Arg	--	--	100	72
Gly-Lys-Val-Arg	--	--	100	6
Asp-Orn-Ser-Arg	200	98	200	84
Asp-His-Ser-Arg	50	-28	100	-14

1/ Percentage inhibition as compared to cultures in the absence of peptide.

NOTE: Cells were cultured at 37°C and 5% CO_2 for 48 hours and then pulsed with 1 µCi ^{3}H-thymidine for 6 hours.

The concentrations of the subject peptides which produce inhibition may occasionally vary from the concentrations listed depending on many factors including age of the cells, culture conditions and other factors.

TABLE 5

PERCENT INHIBITION OF DNA PROLIFERATION OF HUMAN MENINGIOMA TUMOR CELLS IN THE PRESENCE OF THE SUBJECT PEPTIDES

Peptide Sequence	EFc Tumor Cells [Peptide] (μg/ml)	EFc Tumor Cells % Inhib.[1/]	LG Tumor Cells [Peptide] (μg/ml)	LG Tumor Cells % Inhib.[1/]
Lys-NH_2	200	74	200	84
D-Lys-NH_2	200	93	200	96
Lys-Ser	200	88	200	88
Lys-Ser-NH_2	100	65	100	78
desamino-Lys-Ser	200	20	200	25
Ac-Lys-Ser-NH_2	200			
N-CH_3-Lys-D-Ser	100	82	50	-439
Orn-D-Ala	200	88	100	52
Orn-Gly	200	72	200	48
Arg-D-Ala	200	37	100	50
Arg-Sar	200	82	50	50
His-Ser	200	31	200	-10
Lys-Cys	200	91	100	97
Lys-Phe	100	63	200	77
Lys-Tyr	200	46	100	-42
Ala-Lys-Ser	200	90	200	95
Ala-Lys-Gly	50	79	50	72
D-Ala-D-Lys-D-Ser	200	23	200	45
Gly-Lys-Ser	50	-12	50	53
Phe-Lys-D-Ala	200	16	--	--
Lys-Ser-Tyr	100	37	100	-9
Lys-Ser-Ala	200	46	200	58
Lys-Arg-Lys	100	70	50	72
Lys-Val-Lys-NH_2	200	54	200	76
Lys-Ley-Lys-NH_2	200	49	--	--
Asp-Lys-Lys-Arg	100	65	200	79

1/ Percentage inhibition as compared to cultures in the absence of peptide.

NOTE: Cells were cultured at 37°C and 5% CO_2 for 48 hours and then pulsed with 1 μCi H-thymidine for 6 hours.

The concentrations of the subject peptides which produce inhibition may occasionally vary from the concentrations listed depending on many factors including age of the cells, culture conditions and other factors.

EXAMPLE 1

Solution phase synthesis of Lys-Ser salts using t-butyloxycarbonyl protection. L-Serine (157.6 g, 1.5 moles) and sodium bicarbonate (168 g, 2 moles) were dissolved in distilled water (2 l) and treated with a solution of N,N'-bis-t-butyloxycarbonyl-L-lysine N-hydroxysuccinimide ester (444 g, 1 mole) in tetrahydrofuran (2 l). The resulting solution (pH approx. 9) was stirred overnight at room temperature. The pH was then adjusted to 6.5 with 1 N sodium bisulfate and the mixture evaporated to approximately half the volume under reduced pressure. The residue was extracted twice with ether before adjusting the pH to 3.0 (1 N sodium bisulfate) and extracting the product three times with ethyl acetate (2 l, 1 l, 1 l). The combined organic extracts were washed with 5% brine and then dried over sodium sulfate. The solution was then concentrated under reduced pressure to approximately 1.5 l, dicyclohexylamine (199.2 ml, 1 mole) added and the mixture stirred at room temperature for 0.5 hours. The product, N,N'-bis-t-butyloxycarbonyl-L-lysyl-L-serine dicyclohexylammonium salt, crystallized out of the solution and was recrystallized from isopropanol. Yield: 464 g (76%), m.p. 126-129°C, $[\alpha]_D^{25} = +4.48°$ (c = 1, ethanol).

The dicyclohexylammonium salt (73 g) was dissolved in ice-water (800 ml), the pH adjusted to 3 with 1 N sodium bisulfate and the product extracted into ethyl acetate (500 ml, 200 ml, 100 ml). The combined organic extracts were dried over sodium sulfate and the solution evaporated to give N,N'-bis-t-butyloxycarbonyl-L-lysyl-L-serine (46.3 g).

Deprotection to bis-trifluoroacetate salt. N,N'-bis-t-butyloxycarbonyl-L-lysyl-L-serine (17.8 g) was dissolved in dichloromethane (70 ml), anhydrous trifluoroacetic acid (50 ml) added and the mixture stirred at room temperature for 0.5 hours. The solution was evaporated under reduced pressure and the residue triturated three times with dichloromethane and three times with ether to give the bis-trifluoroacetate salt of L-lysyl-L-serine in quantitative yield as a colorless, hygroscopic solid.

Deprotection to dihydrochloride salt. N,N'-Bis-t-butyloxycarbonyl-L-lysyl-L-serine (20 g) was dissolved in an anhydrous solution of hydrogen chloride in ethyl acetate (3.5 N, 200 ml) and the mixture stirred at room temperature for 0.5 hours. The solution was evaporated under reduced pressure and the residue triturated four times with ethér to give the dihydrochloride salt of L-lysyl-L-serine in quantitative yield as a white hygroscopic solid.

Alternately, N,N'-bis-t-butyloxycarbonyl-L-lysyl-L-serine (25 g) was dissolved in dioxane (50 ml) and treated with a solution of hydrogen chloride in anhydrous dioxane (4 N, 250 ml). The mixture was stirred at room temperature for 0.5 hours, evaporated under reduced pressure and the residue triturated four times with ether to give the dihydrochloride salt of L-lysyl-L-serine in quantitative yield as a colorless, glassy hygroscopic solid.

EXAMPLE 2

Solution phase synthesis of Lys-Ser salts using benzyloxycarbonyl protection. Following the general procedure described in Example 1, L-serine, (15.76 g, 0.15 mole) and sodium bicarbonate (25.2 g, 0.3 mole) were dissolved in distilled water (325 ml) and treated with a solution of N,N'-bis-benzyloxycarbonyl-L-lysine N-hydroxysuccinimide ester (51.56 g, 0.1 mole) in tetrahydrofuran (300 ml). After stirring at room temperature overnight, the reaction mixture was concentrated under reduced pressure to approximately half the volume, extracted with ether (2 x 500 ml) and poured, with rapid stirring, into ice-cold hydrochloric acid (1 N, 3.5 l). The white precipitate which separated was quickly extracted into ethyl acetate (3 l) and the organic phase washed with ice-cold 1 N HCl (2 x 1 l), water (3 x 1 l) and dried over magnesium sulfate. The solution was evaporated to dryness under reduced pressure and the solid residue triturated with hexanes to give N,N'-bis-benzyloxycarbonyl-L-lysyl-L-serine as a colorless, hygroscopic solid, m.p. 143-145°C. Yield: 45.03 g.

Conversion to dicyclohexylammonium salt. The product was converted to the dicyclohexylamine salt by dissolving in a minimum volume of ethyl acetate (approx. 7 l) containing methanol (1 l) and treating with 1 equivalent of redistilled dicyclohexylamine (17.9 ml). The crystalline product was filtered, washed with ether, dried and recrystallized from isopropanol. The product was reconverted to the protected dipeptide by neutralizing with aqueous sodium bisulfate, as described in Example 1. Yield: 31.5 g.

Deprotection to acetate salt. N,N'-Bis-benzyloxycarbonyl-L-lysyl-L-serine (3 g) was dissolved in a mixture of methanol (20 ml) and water (20 ml) containing acetic acid (0.35 ml, 2 equivalents) and hydrogenated over 10% palladium on carbon (1 g) at atmospheric pressure overnight. After removing the catalyst by filtration, the solution was concentrated to approximately half the volume under reduced pressure, and the residue lyophilized, to give the acetate salt of L-lysyl-L-serine in quantitative yield as a colorless, hygroscopic solid.

EXAMPLE 3

Automated solid-phase synthesis of Lys-Ser on chloromethylated polystyrene resin. Tert-butyloxycarbonyl-amino acid resins, protected amino acid derivatives, and other peptide synthesis products may be obtained from, e.g., Peninsula Laboratories, Inc., 611 Taylor Way, Belmont, CA 94002, or Bachem, Inc., 3132 Kashiwa Street, Torrance, CA 90505. Tert-butyloxycarbonyl-O-benzyl-L-serine-resin (substitution = 0.85 mmoles/g; 4.0 g) was deblocked and coupled with t-butyloxycarbonyl-ε-benzyloxycarbonyl-L-lysine using an automated synthesizer (Beckman Model 990) programmed with the following protocol:

1. Wash with dichloromethane (50 ml), 3 x 1 minute.

2. Deblock with trifluoroacetic acid (35% v/v in dichloromethane, 50 ml), 1 x 1 minute, 1 x 20 minutes.

3. Wash with dichloromethane (50 ml), 3 x 1 minute; isopropanol (50 ml), 2 x 1 minute; and dichloromethane (50 ml), 3 x 1 minute.

4. Neutralize with triethylamine (10% v/v in dichloromethane, 50 ml), 1 x 1 minute and 1 x 5 minutes.

5. Wash with dichloromethane (50 ml), 4 x 1 minute.

6. Couple with t-butyloxycarbonyl-ε-benzyloxycarbonyl-L-lysine (2.59 g, 6.8 mmoles, 2 equivalents) in dichloromethane (30 ml), together with N,N-dicyclohexylcarbodiimide (0.5 M in dichloromethane, 13.6 ml, 2 equivalents), 1 x 90 minutes.

7. Repeat step 3.

8. Repeat step 1.

9. Repeat step 2.

10. Repeat step 3.

(Note: Steps 1-7 constitute one complete coupling cycle.)

The resin was collected on a sintered glass funnel, washed successively with ethanol and dichloromethane and dried under vacuum. Yield: 4.96 g.

The peptide was cleaved from the resin by treatment with liquid hydrogen fluoride (10 ml per gram of dry resin) in the presence of anisole (1 ml per gram of resin) and 0°C for one hour. After removal of the hydrogen fluoride under vacuum, ether (10 ml per gram of resin) was added, the resin transferred to a sintered glass funnel and washed alternately with ether (4 x 40 ml) and chloroform (3 x 40 ml). The resin was sucked dry and the peptide extracted with 1 N aqueous acetic acid (4 x 30 ml). The combined extracts were lyophilized, redissolved in distilled water (7 ml) and relyophilized

to give 816 mg of the acetate salt of the crude peptide, Lys-Ser.

Analogously Lys-Val-Lys is obtained from tert.-butoxycarbonyl-ε-2-chlorobenzoxy-carbonyl-L-lysine-resin with t-butoxycarbonyl-L-valine and t-butoxycarbonyl-ε-benzoxycarbonyl-L-lysine.

EXAMPLE 4

<u>Purification of Lys-Ser using carboxymethyl cellulose chromatography</u>. The crude peptide salt from Example 3 (2.5 g) was dissolved in distilled water (1 l), the pH adjusted to 5.0-6.5 (1 M aqueous ammonia) and the solution applied to a column (2.5 x 30 cm) of Whatman CM-52 which had been equilibrated with 0.01 M ammonium acetate, pH 5.0-6.5. The column was washed copiously with the equilibration buffer and then eluted with a linear gradient of 0.01 M ammonium acetate, pH 4.5-6.5 (350 ml) to 0.30 M ammonium acetate, pH 6.5 (350 ml). The pH of the column and the starting buffer was adjusted to equal that of the peptide solution before loading. The fractions containing the pure product were combined and lyophilized to give L-lysyl-L-serine as a colorless, hygroscopic solid.

Analogously, Lys-Val-Lys is purified.

EXAMPLE 5

Purification of Lys-Ser using carboxymethyl Sephadex chromatography. Following the general procedure described in Example 4, the crude peptide from Example 2 (2 g) was dissolved in distilled water (1 l), the pH adjusted to 4.5-6.5 (1 M aqueous ammonia) and the solution applied to a column (2.5 x 30 cm) of CM-Sephadex (Pharmacia, Inc.) which had been equilibrated with 0.01 M ammonium acetate, pH 4.5-6.5. The column was then eluted with a linear gradient of 0.01 M ammonium acetate, pH 4.5-6.5 (350 ml) to 0.30 M ammonium acetate, pH 6.5 (350 ml). The pH of the column and the starting buffer was adjusted to equal that of the peptide solution before loading. Fractions containing the pure product were combined and lyophilized to give L-lysyl-L-serine as a colorless, glassy, hygroscopic solid. Analogously, Lys-Val-Lys is purified.

EXAMPLE 6

Purification of Lys-Ser using reverse-phase chromatography on C-18 silica. The crude peptide from Example 1 (5.0 g) was dissolved in a minimum volume of distilled water (approximately 10 ml) and applied to a column (5 x 50 cm) of C-18 silica gel (Separation Technologies, Inc.) which had been previously equilibrated with 0.5% aqueous hydrochloric acid. The column was eluted with the same solvent and the fractions containing the pure product combined and lyophilized to give L-lysyl-L-serine hydrochloride salt as a colorless, hygroscopic, glassy solid.
Analogously, Lys-Val-Lys is purified.

EXAMPLE 7

Purification of Lys-Ser using Sephadex G-10 chromatography. The peptide from Example 2 (1.0 g) was dissolved in a minimum volume of distilled water (5 ml) and applied to a column (2.5 x 100 cm) of Sephadex G-10 (Pharmacia) which had been equilibrated with 3% aqueous acetic acid. The column was eluted with the same solvent and the fractions which contained the pure product combined and lyophilized to give L-lysyl-L-serine acetate salt as a colorless, glassy, hygroscopic solid.

Analogously, Lys-Val-Lys is purified.

EXAMPLE 8

Automated solid-phase synthesis of Lys-NH_2 on benzhydrylamine resins. N-t-butyloxycarbonyl-ε-benzyloxycarbonyl-L-lysine (2.31 g, 6.08 mmole) was coupled to preswollen benzhydrylamine resin (1% cross-linked, substitution = 0.76 meq. amine per gram) in dichloromethane (30 ml) using a solution of dicyclohexylcarbodiimide in dichloromethane (0.5 M, 12.2 ml; 2 equivalents) in an automated synthesizer, as described in Example 3. The mixture was stirred for 90 minutes, the resin thoroughly washed (see protocol in Example 3, steps 7-10), transferred to a sintered glass funnel, washed successively with ethanol and dichloromethane and dried under vacuum. Yield: 4.7 g.

The crude peptide, Lys-NH_2, was cleaved from the resin using liquid hydrogen fluoride, following the procedure described in Example 3. Yield: 693 mg.

Analogously, Lys-Val-Lys-NH_2 is prepared.

Peptide amides may be synthesized by an identical procedure, using 4-methylbenzhydrylamine resin in place of benzhydrylamine resin.

EXAMPLE 9

Synthesis of N^{α}-acetyl-Lys-Ser (Ac-Lys-Ser) by acetylation of a peptide resin. The dipeptide was assembled by the solid-phase method, as described in Example 3, starting with 2.9 g of t-butyloxycarbonyl-O-benzyl-L-serine resin (substitution = 0.65 mmole/g). After cleavage of the N-terminal t-butyloxycarbonyl group from the dipeptide-resin, it was acetylated using the following protocol:

1. Wash with dichloromethane (50 ml), 3 x 1 minute; ethanol (50 ml), 2 x 1 minute; and dichloromethane (50 ml), 3 x 1 minute.
2. Neutralize with triethylamine (10% in dichloromethane, 50 ml), 1 x 1 minute and 1 x 5 minutes.
3. Acetylate by adding dichloromethane (40 ml), followed by acetic anhydride (1.9 ml per mmole of peptide-resin) and triethylamine (2.8 ml per mmole of peptide-resin) for 20 minutes.
4. Repeat step 1.

The resin was collected on a sintered glass funnel, washed successively with ethanol and dichloromethane and dried under vacuum.

The peptide was cleaved from the resin using liquid hydrogen fluoride at 0°C, as described in Example 3 and purified by chromatography on C-18 silica, as described in Example 6 to give N^{α}-acetyl-L-lysyl-L-serine acetate as a colorless, glassy hygroscopic solid. Yield: 304.7 mg.

EXAMPLE 10

Synthesis of D-Lysine N-ethylamide (D-Lys-$NHCH_2CH_3$) via aminolysis of an amino acid resin. N^{α}-t-Butyloxycarbonyl-ε-benzyloxycarbonyl-D-lysine-resin (substitution = 0.67 mmole/g, 1.5 g) was

suspended in dimethylformamide (15 ml) and anhydrous ethylamine gas bubbled slowly through the solution until saturation was achieved and the mixture stored in a stoppered flask for 24 hours at room temperature. The resin was removed by filtration, washed with dimethylformamide and the filtrate evaporated under reduced pressure. The residue was triturated with ether to give crude N-t-butyloxycarbonyl-ε -benzyloxycarbonyl-D-lysine N-ethylamide as a white solid. Yield: 0.4 g.

The product was deprotected using liquid hydrogen fluoride (5 ml) at 0°C in the presence of anisole (2 ml), as described in Example 3. Purification was accomplished by chromatography on Sephadex G-10, as described in Example 7 to give D-lysine N-ethylamide as a white, hygroscopic solid. Yield: 70 mg.

EXAMPLE 11

General method for the preparation of t-butyloxycarbonyl amino acid resins. Chloromethylated polystyrene beads (Biobeads SX-1, Bio-Rad; 1% crosslinked; substitution = 1.25 milliequivalents Cl/g) are added to a solution of the t-butyloxycarbonyl-amino acid derivative (1 equivalent) in dimethylsulfoxide (3 ml per gram of resin) in a round bottom flask, and the mixture treated with a solution of potassium t-butoxide (1 equivalent) in dimethylsulfoxide (1.5 ml per gram of resin; centrifuged to remove insolubles). The mixture is heated with agitation at 80°C using an oilbath for two hours and then allowed to stand at room temperature overnight. The resin is transferred to a sintered glass funnel, washed with dimethylsulfoxide

(3 times), alternately with methanol and dichloromethane (3 times each) and finally with methanol (2 times) and dried under vacuum. Degree of substitution is calculated from the weight gain of the resin.

EXAMPLE 12

Acetate salts of the peptides listed in Table 6 were prepared and purified under the protocols described in the foregoing examples, using the materials and protocols specified in the table. Peptide R_f and specific rotation data are given in Table 7.

EXAMPLE 13

Solution-phase synthesis of Lys-Val-Lys acetate salt. Tert-butyloxycarbonyl-L-valine (22.8 g, 0.105 moles) is dissolved in dry tetrahydrofuran (400 ml), the solution cooled to -15°C and treated with N-methylmorpholine (11.0 ml, 0.1 mole), followed by isobutyl chloroformate (13.0 ml, 0.1 mole). The mixture is stirred at -15° for 10 minutes and then treated with a precooled colution of ε-benzyloxycarbonyl-L-lysine benzyl ester hydrochloride (40.65 g, 0.1 mole) in tetrahydrofuran (200 ml) and dimethylformamide (50 ml). The reaction mixture is allowed to warm slowly to room temperature and stirred overnight. The solvent is evaporated under reduced pressure, the residue redissolved in ethyl acetate (2 l) and washed with 1 N hydrochloric acid (3 x 500 ml), 5% sodium chloride (3 x 500 ml), 5% sodium bicarbonate

(3 x 500 ml) and finally with water (3 x 500 ml). The organic phase is dried over magnesium sulfate, concentrated under reduced pressure and crystallization of the product induced by addition of hexane to the cloud point. Yield: 46.7 g (82%).

Tert-butyloxycarbonyl-L-valyl-ε-benzyloxycarbonyl-L-lysine benzyl ester from the previous step (46.7 g) is deprotected by dissolving in dichloromethane (250 ml) and treating with cold (0°C) trifluoroacetic acid (150 ml) for 30 minutes. The solvent is evaporated under reduced pressure and the residue triturated several times with ether to give L-valyl-ε-benzyloxycarbonyl-L-lysine benzyl ester trifluoroacetate salt in quantitative yield, which may be used without further purification.

N,N-bis-benzyloxycarbonyl-L-lysine (35.6 g, 0.086 mole) is dissolved in tetrahydrofuran (350 ml), the solution cooled to -15°C and treated with N-methylmorpholine (9.02 ml, 0.082 mole) followed by isobutyl chloroformate (10.64 ml, 0.082 mole). The mixture is stirred for 10 minutes and then treated with a pre-cooled solution of L-valyl-ε-benzyloxycarbonyl-L-lysine benzyl ester trifluoroacetate (46.4 g, 0.082 mole) in tetrahydrofuran (200 ml) and dimethylformamide (50 ml), followed by N-methylmorpholine (9.02 ml, 0.082 mole). The mixture is allowed to warm slowly to room temperature and stirred overnight. The product is worked up as described above to give N,N-bis-benzyloxycarbonyl-L-lysyl-L-valyl-ε-benzyloxycarbonyl-L-lysine benzyl ester. Yield: 49.0 g (78%).

The protected tripeptide (49.0 g) is dissolved in glacial acetic acid (1 l) and hydrogenated over palladium on carbon (10%, 10 g) overnight at atmospheric pressure. The catalyst is removed by filtration, the filtrate evaporated and the residue redissolved and lyophilized to give L-lysyl-L-valyl-L-lysine acetate salt in quantitative yield (37.0 g).

Purification may be achieved by the methods of Examples 4 through 7.

TABLE 6

Product Peptide	S*	Starting Resin	Coupling Cycle Reagents	P**
D-Lys-NH_2	8	Boc-ε-Cbz-D-Lys benzhydrylamine	--	7
Lys-Ser-NH_2	8	Boc-OBzl-L-Ser benzhydrylamine	Boc-ε-Cbz-L-Lys	4,7
D-Lys-Ser	3	Boc-OBzl-L-Ser	Boc-ε-Cbz-D-Lys	4,7
Desamino-Lys-Ser	3	Boc-OBzl-L-Ser	t-Boc-des-α-amino-Lys	6
Orn-D-Ala	3	Boc-D-Ala	Boc-δ-Cbz-L-Orn	4,7
Arg-D-Ala	3	Boc-D-Ala	Boc-N^g-tosyl-L-Arg	4,7
His-Ser	3	Boc-OBzl-L-Ser	Boc-N^{im}-tosyl-L-His	7
Lys-Cys	3	Boc-δ-MeCbz-Cys	Boc-ε-Cbz-L-Lys	4,7
Orn-Gly	3	Boc-Gly	Boc-δ-Cbz-L-Orn	4,7
Lys-Phe	3	Boc-L-Phe	Boc-ε-Cbz-L-Lys	6
Lys-Asp	3	Boc-β-Cbz-L-Asp	Boc-ε-Cbz-L-Lys	7
Lys-Pro	3	Boc-L-Pro	Boc-ε-Cbz-L-Lys	4,7
Ac-Lys-Ser-NH_2	8,9	Boc-OBzl-L-Ser benzhydrylamine	Boc-ε-Cbz-L-Lys	6 1)
N-Me-Lys-D-Ser	3	Boc-OBzl-L-Ser	Boc-N^{α}-Me-ε-Cbz-L-Lys (threefold excess)	6,7
Ala-Lys-Ser	3	Boc-OBzl-L-Ser	Boc-ε-Cbz-L-Lys; Boc-L-Ala	6 7
D-Ala-D-Lys-D-Ser	3	Boc-OBzl-D-Ser	Boc-ε-Cbz-D-Lys; Boc-D-Ala	7
Val-Lys-Ser	3	Boc-OBzl-L-Ser	Boc-ε-Cbz-L-Lys; Boc-L-Val	6 2)
Phe-Lys-D-Ala	3	Boc-D-Ala	Boc-ε-Cbz-L-Lys; Boc-L-Phe	6
Lys-Ser-Tyr	3	Boc-OBzl-L-Tyr	Boc-OBzl-L-Ser; Boc-ε-Cbz-L-Lys	6
Phe-Lys-Ser-Tyr	3	Boc-OBzl-L-Tyr	Boc-OBzl-L-Ser; Boc-ε-Cbz-L-Lys; Boc-L-Phe	6 1)

/Table 6 continues an next page/

/Table 6, continued/

Lys-Glu-Lys	3	Boc-ε-Cbz-L-Lys	Boc-γ-Bzl-L-Glu; Boc-ε-Cbz-L-Lys	7
Lys-Gln-Lys	3	Boc-ε-Cbz-L-Lys	Boc-X-L-Gln; Boc-ε-Cbz-L-Lys	7
Lys-Arg-Lys	3	Boc-ε-Cbz-L-Lys	Boc-N^g-tosyl-L-Arg; Boc-ε-Cbz-L-Lys	7
Lys-His-Lys	3	Boc-ε-Cbz-L-Lys	Boc-N^{im}-tosyl-L-His; Boc-ε-Cbz-L-Lys	7
Lys-D-Ala-Lys	3	Boc-ε-Cbz-L-Lys	Boc-D-Ala; Boc-ε-Cbz-L-Lys	4,7
Lys-Leu-Lys-NH_2	8	Boc-ε-Cbz-L-Lys benzhydrylamine	Boc-L-Leu; Boc-ε-Cbz-L-Lys	7
Lys-Pro-Lys-NH_2	8	Boc-ε-Cbz-L-Lys benzhydrylamine	Boc-L-Pro; Boc-ε-Cbz-L-Lys	7
Lys-Tyr-Lys-NH_2	8	Boc-ε-Cbz-L-Lys benzhydrylamine	Boc-OBzl-L-Tyr; Boc-ε-Cbz-L-Lys	6[3)]
Lys-Ser-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-OBzl-L-Ser; Boc-ε-Cbz-L-Lys	7
Lys-Thr-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-OBzl-L-Thr Boc-ε-Cbz-L-Lys	7
Arg-Gly-Lys	3	Boc-ε-Cbz-L-Lys	Boc-Gly; Boc-N^g-tosyl-L-Arg	4,7
Arg-Ala-Lys	3	Boc-ε-Cbz-L-Lys	Boc-L-Ala; Boc-N^g-tosyl-L-Arg	4,7
Arg-Asp-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-β-Bzl-L-Asp; Boc-N^g-tosyl-L-Arg	7
Ala-Lys-Val-Lys-NH_2	8	Boc-ε-Cbz-L-Lys benzhydrylamine	Boc-L-Val; Boc-ε-Cbz-L-Lys; Boc-L-Ala	6[4)]
Arg-Lys-Ser-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-OBzl-L-Ser; Boc-ε-Cbz-L-Lys; Boc-N^g-tosyl-L-Arg	6[1)]
Ac-Asn-Lys-Ser-Arg	3,9	Boc-N^g-tosyl-L-Arg	Boc-OBzl-L-Ser; Boc-ε-Cbz-L-Lys; Boc-Xan-L-Asn	7

/Table 6 continues on next page/

[Table 6 continued]

Gly-Asp-Lys-Ser-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-OBzl-L-Ser; Boc-ε-Cbz-L-Lys; Boc-β-Bzl-L-Asp; Boc-Gly	7
Asp-Lys-Lys-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-ε-Cbz-L-Lys; Boc-ε-Cbz-L-Lys; Boc-β-Bzl-L-Asp	7
Asp-Lys-Ile-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-L-Ile; Boc-ε-Cbz-L-Lys; Boc-β-Bzl-L-Asp	7
Gly-Lys-Val-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-L-Val; Boc-ε-Cbz-L-Lys; Boc-Gly	6[1)]
Asp-Orn-Ser-Arg	3	Boc-N^g-tosyl-L-Arg	Boc-OBzl-L-Ser; Boc-δ-Cbz-L-Orn Boc-β-Bzl-L-Asp	7
Asp-Lys-Ser-His	3	Boc-N^{im}-tosyl-L-His	Boc-OBzl-L-Ser; Boc-ε-Cbz-L-Lys; Boc-β-Bzl-L-Asp	7

* S refers to the synthesis protocol followed, as described in the Example number(s) given in this column.

** P refers to the purification protocol followed, as described in the Example number(s) given in this column. Where two protocols are listed, these were utilized in the order listed.

1) 0-60% acetonitrile gradient in 0.5% aqueous HCl used for elution.

2) 0-60% acetonitrile gradient in 0.1 M ammonium acetate, pH 4.5, used for elution.

3) 0-30% acetonitrile gradient in 0.5% aqueous HCl used for elution.

4) 0-50% acetonitrile gradient in 0.5% aqueous HCl used for elution.

Note: The following abbreviations apply to Table 6: Boc, tert-butyloxycarbonyl; Cbz, benzyloxycarbonyl; OBzl, O-benzyl; MeBzl, 4-methylbenzyl; Bzl, benzyl; Me, methyl; Xan, xanthydryl.

TABLE 7

PEPTIDE R_f AND SPECIFIC ROTATION DATA

Product Peptide	R_f 1/	[α](c) 2/
Lys-Ser	0.15	+ 20.42 (4.26)
Lys-NH_2	0.15	+ 16.21 (4.38)
D-Lys-NH_2	0.14	- 13.39 (3.66)
Lys-Ser-NH_2	0.16	+ 20.32 (4.92)
D-Lys-Ser	0.15	- 42.54 (4.00)
Desamino-Lys-Ser	0.34	+ 2.36 (6.78)
Orn-D-Ala	0.15	+ 64.36 (5.36)
Arg-D-Ala	0.28	+ 56.15 (6.50)
His-Ser	0.16	- 31.44 (3.60)
Lys-Cys	0.18	+ 9.70 (3.40)
Orn-Gly	0.16	+ 44.20 (4.14)
Lys-Phe	0.37	+ 1.46 (3.20)
Lys-Asp	0.20	+ 24.11 (11.60)
Lys-Pro	0.14	- 55.60 (4.04)
Ac-Lys-Ser-NH_2	0.26	- 35.50 (4.00)
N-Me-Lys-D-Ser	0.13	+ 51.23 (4.06)
Ala-Lys-Ser	0.15	- 17.25 (4.00)
D-Ala-D-Lys-D-Ser	0.10	+ 17.88 (3.41)
Val-Lys-Ser	0.16	+ 0.61 (14.8)
Phe-Lys-D-Ala	0.32	+ 16.67 (4.50)
Lys-Ser-Tyr	0.08	+ 18.78 (4.10)
Phe-Lys-Ser-Tyr	0.05	+ 7.01 (4.28)

[Table 7 continues on next page]

1/ TLC analysis on Silica Gel 60 plates (MERCK) in the solvent system n-butanol:acetic acid:water:ethyl acetate (1:1:1:1).

2/ Specific rotation [α] measured in water at the concentration (in mg/ml) indicated in parentheses.

/Table 7, continued/

Lys-Val-Lys	0.10	- 4.78	(3.56)
Lys-Val-Lys-NH_2	0.09	-10.78	(4.64)
Lys-Glu-Lys	0.09	+ 1.84	(5.42)
Lys-Gln-Lys	0.06	nd	
Lys-Arg-Lys	0.07	+ 2.57	(15.8)
Lys-His-Lys	0.03	+ 9.86	(5.36)
Lys-D-Ala-Lys	0.07	+45.03	(6.44)
Lys-Leu-Lys-NH_2	0.09	- 5.46	(4.60)
Lys-Pro-Lys-NH_2	0.05	-41.67	(5.04)
Lys-Tyr-Lys-NH_2	0.14	+30.88	(4.34)
Lys-Ser-Arg	0.06	nd	(
Lys-Thr-Arg	0.09	- 1.36	(5.88)
Arg-Gly-Lys	0.08	+10.38	(5.20)
Arg-Ala-Lys	0.10	- 4.59	(3.92)
Arg-Asp-Arg	0.14	+ 3.95	(18.0)
Ala-Lys-Val-Lys-NH_2	0.07	-45.57	(4.06)
Arg-Lys-Ser-Arg	0.01	nd	
Ac-Asn-Lys-Ser-Arg	0.13	nd	
Gly-Asp-Lys-Ser-Arg	0.03	-32.50	(32.4)
Asp-Lys-Lys-Arg	0.03	nd	
Asp-Lys-Ile-Arg	0.10	nd	
Gly-Lys-Val-Arg	0.12	nd	
Asp-Orn-Ser-Arg	0.06	-20.49	(3.49)
Asp-Lys-Ser-His	0.03	nd	

In addition to the peptides mentioned above, the following preferred compounds can be prepared in analogy to the Examples:

Ac-Asn-Lys-Val-Arg
Ac-Lys-Ser-NH-CH_3
Ala-Lys-D-Ala
Ala-Lys-Ala-Lys
Ala-Lys-Gln
Ala-Lys-Gly-Lys
Ala-Lys-Phe
Ala-Lys-Pro
Ala-Lys-Sar
Ala-D-Lys-D-Ser
Ala-Lys-Val-Arg
Ala-Lys-Thr-Lys
Ala-Orn-Ala
Ala-Orn-D-Ala
Ala-Orn-Ser
Ala-D-Orn-Ser
Arg-Ala
Arg-Gln
Arg-Gly
Arg-Pro
Arg-Ser
Arg-Ser-Arg
Arg-Ser-Lys
Arg-Thr-Lys
Asp-Arg-Ser-Arg
Asp-Lys-Ala-Arg
Asp-Lys-Ser-Arg-NH_2
Asp-Lys-Ser-Lys
Des-α-amino-Lys-Ala
Des-α-amino-Lys-Ala-Lys
Des-α-amino-Lys-Arg-Lys
Des-α-amino-Lys-Asp-Lys
Des-α-amino-Lys-Cys
Des-α-amino-Lys-Gln
Des-α-amino-Lys-Gln-Lys
Des-α-amino-Lys-Gly
Des-α-amino-Lys-Gly-Lys
Des-α-amino-Lys-His-Lys
Des-α-amino-Lys-HomoSer
Des-α-amino-Lys-Lys-Lys
Des-α-amino-Lys-Lys-Thr-Glu-Thr
Des-α-amino-Lys-NH_2
Des-α-amino-Lys-Pro
Des-α-amino-Lys-Ser-Tyr
Des-α-amino-Lys-Thr-Glu
Des-α-amino-Lys-Thr-Glu-Thr
Des-α-amino-Lys-Thr-Glu-Thr-Gln-Glu-Lys
Des-α-amino-Lys-Thr-Lys
Des-α-amino-Lys-Val-Lys
Des-α-amino-Lys-Val-Lys-NH_2
Gln-Glu-Lys
Gln-Glu-Lys-Asn-Pro-Leu-Pro
Gln-Lys-Ser
Glu-Lys-Asn-Pro-Leu-Pro
Glu-Lys-Ser-Arg
Glu-Thr
Glu-Thr-Gln-Glu-Lys
His-Ala
His-Gly
HomoLys-Gly
HomoLys-Ser
Leu-Lys-Lys-Thr-Glu-Thr
Lys-Ala
Lys-D-Ala
Lys-Ala-Lys

Lys-Ala-Tyr
Lys-Asp-Lys
Lys-Gln-Lys-NH_2
Lys-Glu-Lys-NH_2
Lys-Gly
Lys-Gly-Lys
Lys-Gly-Lys-NH_2
Lys-Gly-Tyr
Lys-Lys-Lys
Lys-Lys-Thr
Lys-Lys-Thr-Glu-Thr
Lys-Phe-Lys-NH_2
Lys-Ser-Lys
D-Lys-Ser-NH_2
Lys-Ser-NH-CH_3
D-Lys-Ser-NH-CH_3
Lys-Ser-NH-C_2H_5
D-Lys-Ser-NH-C_2H_5
Lys-Thr-Glu
Lys-Thr-Glu-Thr
Lys-Thr-Glu-Thr-Gln-Glu-Lys
Lys-Thr-Lys
N-Me-Lys-Ser
N-Me-Lys-Ser-NH_2
N-Me-Lys-Ser-NH-CH_3
N-Me-Lys-Ser-NH-C_2H_5
Orn-Ser
D-Orn-Ser
Orn-Ser-NH_2
D-Orn-Ser-NH_2
Orn-Ser-NH-CH_3
D-Orn-Ser-NH-CH_3
Orn-Ser-NH-C_2H_5
D-Orn-Ser-NH-C_2H_5
Phe-Lys-Gly
Phe-Lys-Ser
Sar-Lys-Ser
Sar-D-Lys-Ser
Thr-Gln-Glu-Lys
Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro
Thr-Glu-Thr
Thr-Glu-Thr-Gln-Glu-Lys
Val-Lys-Thr-Arg.

In the syntheses described in the foregoing examples, the solvents and reagents were invariably of the highest, commercially-available grade and were used without purification, except in the following cases:

Triethylamine was dried over solid potassium hydroxide, decanted and distilled from ninhydrin (1 g per liter of triethylamine) at atmospheric pressure.

Trifluoroacetic Acid was distilled from phosphorus pentoxide at atmospheric pressure.

Dicyclohexylamine was dried over solid potassium hydroxide and distilled under vacuum.

Dioxane was dried over metallic sodium pieces (freshly cut) and distilled at atmospheric pressure.

Dimethylformamide was dried over solid potassium hydroxide, decanted and distilled from ninhydrin (1 g per liter of solvent) under vacuum.

Pyridine was dried over potassium hydroxide, decanted and distilled from ninhydrin (1 g per liter of solvent) at atmospheric pressure.

EXAMPLE 14

Methods for assessing purity of intermediates and final products. The purity of intermediates, column fractions (from purifications) and final products was assessed by a combination of techniques. Thin layer chromatography (TLC) was carried out on glass-backed Silica Gel 60 plates (Merck) using the following solvent systems:

1. n-butanol:acetic acid:water:pyridine (30:6:20:24);

2. n-butanol:acetic acid:water:ethyl acetate (1:1:1:1); and

3. isopropanol:ammonia (3%) (1:1).

Products were visualized by spraying the plates with ninhydrin (1% solution in ethanol) or chlorine peptide spray (1% solution of t-butyl hypochlorite in cyclohexane, followed by a 1% solution of o-toluidine in 10% acetic acid).

High performance liquid chromatography (HPLC) was carried out on C-18 reverse-phase columns (5 μm particle size, 4.6 mm i.d. x 25 cm) using gradients of acetonitrile in 5 mM aqueous hexanesulfonic acid or octanesulfonic acid. Flow-rates were typically 1 ml/min., chromatograms were monitored at 215 nm and 10-20 μg loads of samples were injected.

High voltage electrophoresis was carried out in pyridine:acetic acid:water (10:0.4:90) buffers on Whatman 3MM paper at 2 kV. Products were visualized by spraying the chromatograms with ninhydrin (1% solution in ethanol).

Final, purified peptide products were packaged under sterile conditions in vials containing 1 ml of 0.01M phosphate buffer, pH 7.4, per milligram of peptide and lyophilized to a dry powder for storage.

EXAMPLE 15

Murine Mixed Lymphocyte Response Assay Procedure. The murine MLR assay procedure is described in Dutton, R.W., J. Exp. Med., 123:655-671 (1966). The spleen is removed from mice of two different strains, and the spleen cells from each strain are dis-bursed and separately suspended in human serum albumin in RPMI-1640 (2% HSA-RMPI, Irvine Scientific). The cell suspensions are

then centrifuged at 400 G for ten minutes at 15°C, and the cell pellets resuspended in 2% HSA-RPMI to a total volume of 50 ml. The cell concentration is ascertained using, e.g., a hemacytometer. Cell viability, as measured by trypan blue dye exclusion, should be at least 95%. A stimulator cell suspension is prepared by incubating the cells of one strain with 25 μg mitomycin C per ml for 30-60 minutes at 37°C or irradiation with a total of 3200 roentgens in order to inhibit DNA synthesis. After washing the stimulator cells three times with 2% HSA-RPMI, both the stimulator cells and the untreated responder cells are suspended in KC 2000 (KC Biologicals) to a concentration of 4×10^6 cells/ml. Equal portions of each suspension are then combined, and a control group is prepared in a microtiter plate well by transferring 0.1 ml aliquots each of the cell mixture and KC 2000. An experimental group is prepared by transferring to a microtiter plate well 0.1 ml of the cell mixture and 0.1 ml of the test peptide diluted to twice the experimental concentration. The microtiter plates are incubated in a humidified 37°C incubator with an atmosphere of 5% CO_2 for seven days, whereupon 1 μCi ^{3}H-thymidine is added in 0.025 ml/well. The control and experimental groups are incubated for six hours and then harvested using a 12-channel SCATRON cell harvester or the equivalent. After the filter pods have air dried, the radioactivity count due to incorporated ^{3}H-thymidine is ascertained in a LKB minivial scintillation counter using 3 ml Cytoscint (Westchem). The amount of cell-associated radioactivity is used to estimate the degree to which the responding cells in the experimental and control groups are stimulated to proliferate in the presence and in the absence of the subject peptide.

EXAMPLE 16

Human Mixed Lymphocyte Response Assay Procedure. The human MLR assay is discussed in "Lymphocyte Transformation in Cultures of Mixed Leukocytes," Lancet 1:1184-1186 (1965), and in Walthe, W.I. et al. (Weir, D.M., ed.), Handbook of Experimental Immunology (3d ed.), pp. 26.1-26.10, Blackwell Scientific Publications (London 1978). In this assay, venous blood is extracted from two unrelated human subjects (A and B) in sterile syringes containing 0.15 ml heparin (10,000 units/ml) per 60 cc blood. The mononuclear cells are isolated by centrifuging 40 ml of a 1:1 mixture of blood and sterile RPMI-1640 (Irvine Scientific), underlaid with 8 ml Ficoll-Paque (Pharmacia Fine Chemicals), for 30 minutes at 400 G and 15°C. The plasma is discarded and the mononuclear cell band adjusted to 40 ml with 2% human serum albumin in RPMI-1640 (2% HSA-RPMI, Irvine Scientific). The cell mixture is centrifuged for 8 minutes at 40 G and 15°C, residual plasma discarded, and the cell band rediluted and recentrifuged as immediately above. The cell pellet is resuspended in 10.0 ml KC 2000 (KC Biologicals), and the mononuclear cells counted using a Coulter counter or the equivalent. Stimulator cells (A* and B*) are prepared by treating 20 X 10^6 mononuclear cells with irradiation or mitomycin C as described above in the murine MLR assay procedure. Responder cells (A and B) are prepared by washing the untreated cells once with 2% HSA-RPMI. Both responder and stimulator cells are adjusted to a final concentration of 2 X 10^6 mononuclear cells/ml in KC 2000. In a typical one way human MLR assay, experimental and control groups are assayed by incubating equal-volume mixtures of, e.g., responder cells A and stimulator cells B* for seven days with and without the subject

peptide, as described in the murine MLR procedure. ^{3}H-thymidine is then added to each sample, followed by incubation for six hours, harvesting, and counting of the incorporated labelled DNA in the newly replicated cells as in the murine assay.

EXAMPLE 17

Antigen-Specific T Cell Proliferation Assay Procedure. The antigen-specific T-cell proliferation assay consists of injecting a live mouse with a specific antigen for a period sufficient to allow sensitization to the antigen, culturing the sensitized lymph node cells, and then reexposing the lymph node cells to the antigen after treatment with the subject peptide. Following an appropriate incubation period, the amount of immunoresponsive T-cell proliferation is measured using labelled-DNA uptake methods, e.g., tritiated thymidine incorporation, thus providing a measure of the immunoregulatory activity of the subject peptide with respect to a specific antigen. The data in Table 2 reflect peptide-induced inhibition of T-cell proliferation in response to the antigen ovalbumin. Similar assays may be performed using such antigens as bovine serum albumin, human IgG, sheep, chicken or bovine erythrocytes, or haptens such as dinitrophenol.

The antigen-specific T cell proliferation assay is described in "Antigen Induced Proliferation Assay for Mouse T-Lymphocyte Response to Monovalent Antigens," Eur. J. Immuno. 8:112-118 (1978). A mouse is injected subcutaneously at the base

of the tail with 0.1 mg ovalbumin in 0.05 ml Complete Freund's Adjuvant. After seven days, the mouse is sacrificed and its superficial inguinal lymph nodes removed aseptically. Excess fat is removed, and the lymph node cells are dispersed into 15 ml of 2% human serum albumin (HSA) in phosphate-buffered saline (PBS) by, e.g., gentle teasing between the frosted surfaces of two sterile microscope slides. The dispersed single cells are then gently resuspended three to five times using a sterile plastic pipet, transferred to a sterile centrifuge tube, and the supernatent transferred to a new centrifuge tube, free of settled debris, after three minutes. The single cell suspension is centrifuged for ten minutes at 400 G and 15°C, and the pellet resuspended and tested for cell viability by measuring the exclusion of trypan blue dye (0.5% solution, 0.020 ml) from cells of the cell suspension (0.100 ml). The cells should measure at least about 95% viable. The cells are then washed twice in 2% HSA-PBS and resuspended at approximately 8 x 10^6 lymph node cells/ml in Complete Click's Medium. The cell count is determined and the lymph node cell solution diluted to a final concentration of 4 x 10^6 cells/ml with Complete Click's Medium. 0.1 ml aliquots of the culture suspension are transferred into microtiter plate wells, and 0.1 ml of the subject peptide in Complete Click's Medium in the concentrations specified in Table 2 is added to the experimental groups. Control groups receive Complete Click's Medium. After at least 15 minutes, 100 μg/ml of ovalbumin is added to groups with and without the subject peptide. Control groups are prepared without antigen and with 100 μg/ml of anti-

gens unrelated to ovalbumin (e.g., human IgG) or with mitogens such as concanavalin A, phytohemagglutinen, and polkweed mitogen. The plates are incubated at 37°C and 5% CO_2. On day four to day five, 1 μCi of ^{3}H-thymidine is added in 0.025 ml/well, and the plates are incubated for 24 hours before harvesting and counting as described with respect to the murine MLR assay procedure.

EXAMPLE 18

Leukemia and Tumor Cell Proliferation Assay. Mouse leukemia/lymphoma and human tumor cell lines are available from, e.g., American Type Culture Collection, 12301 Parklawn Drive, Rockville MD 20852-1776. The individual cells are gently and sterilely dispersed to suspension in PBS. Cell viability is ascertained using the trypan blue dye exclusion method described in Example 16, and should be at least about 85%. The cells are washed 2-3 times in PBS and then twice in RPMI 1640. The cells are then resuspended in a sufficient quantity of approximately 10% FCS-RPMI or HSA-RPMI to yield a final cell concentration of 1 x 10^6 cells/ml. 0.1 ml aliquots of the tumor cell suspension are transferred to microtiter plate wells. 0.1 ml aliquots of appropriately-diluted peptide in RPMI 1640 are added as indicated in Tables 4 and 5. After incubation for 48 hours at 37°C and 5% CO_2, the cells are pulsed for six hours with 1 μCi ^{3}H-thymidine, harvested, and counted as described in the foregoing examples.

General procedures relating to the measurement of neoplastic cell proliferation may be found in Sample et al., Clin. Exp. Immunol. 9:419 (1971) and in Shellekens et al., Clin. Exp. Immunol. 3:571 (1968).

C L A I M S :

1. Use of a peptide of formula I

$$A-X-(B-Y)_n-C \qquad \text{I}$$

wherein

X and Y are residues of amino acids or amino acid derivatives with positively charged side chains,

A and C are substituents that preserve or augment the immunoregulatory activity of the peptide,

B is a residue of an amino acid or amino acid derivative that preserves or augments the immunoregulatory activity of the peptide, and

n is 0 or 1,

for the manufacture of a medicament for regulating immune system responses.

2. Use of a peptide of formula I for the manufacture of a medicament for suppressing autoimmune disease responses.

3. Use of a peptide of formula I for the manufacture of a medicament for suppressing organ transplantation rejection responses.

4. Use of a peptide of formula I for the manufacture of a medicament for suppressing neoplastic cell growth.

5. Use of a peptide of formula I for the manufacture of a medicament for suppressing T-lymphocyte proliferation in mixed lymphocyte response cultures or in antigen-specific T-lymphocyte proliferation assay cultures.

6. A peptide of formula I.

7. A peptide of any of formulae Ia, Ib, Iaa to Iam, Iama to Iamz, Ian to Iat, Iba to Ibz or Ibza to Ibzo as defined above.

8. A peptide selected from the group consisting of Ala-Lys-Ser, Ala-D-Lys-D-Ser, Arg-Asp-Arg, Asp-Lys-Ser, Des-α-amino-Lys-Ala-Lys, Des-α-amino-Lys-Arg-Lys, Des-α-amino-Lys-Asp-Lys, Des-α-amino-Lys-Cys, Des-α-amino-Lys-Gln, Des-α-amino-Lys-Gln-Lys, Des-α-amino-Lys-Gly-Lys, Des-α-amino-Lys-His-Lys, Des-α-amino-Lys-HomoSer, Des-α-amino-Lys-Lys-Lys, Des-α-amino-Lys-Lys-Thr-Glu-Thr, Des-α-amino-Lys-Pro, Des-α-amino-Lys-Ser, Des-α-amino-Lys-Ser-Tyr, Des-α-amino-Lys-Thr-Glu, Des-α-amino-Lys-Thr-Glu-Thr, Des-α-amino-Lys-Thr-Glu-Thr-Gln-Glu-Lys, Des-α-amino-Lys-Thr-Lys, Des-α-amino-Lys-Val-Lys, Des-α-amino-Lys-Val-Lys-NH_2, Gln-Glu-Lys, Glu-Lys-Asn-Pro-Leu-Pro, Glu-Thr-Gln-Glu-Lys, Gly-Lys-Ser, HomoLys-Gly, HomoLys-Ser, Leu-Lys-Lys-Thr-Glu-Thr, Lys-Ala-Tyr, Lys-Arg-Lys, Lys — Cys, Lys-Gln, Lys-Gln-Lys, Lys-Gly-Tyr, Lys-His-Lys, Lys-HomoSer, Lys-Lys-Thr-Glu-Thr, Lys-Thr-Glu, Lys-Thr-Glu-Thr, Lys-Thr-Lys, Lys-Val-Lys, Lys-Val-Lys-NH_2, Orn-Ala, Thr-Gln-Glu-Lys, Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro, Thr-Glu-Thr, and Val-Lys-Ser.

9. A peptide selected from the group consisting of Ac-Lys-Ser, Ac-Lys-Ser-Lys, Ac-Lys-Ser-NH_2, Ala-Lys-Gly, D-Ala-D-Lys-D-Ser, Ala-Lys-Ser-Arg, Ala-Lys-Val-Lys-NH_2, Arg-D-Ala, Arg-Ala-Lys, Arg-Gly-Lys, Arg-Lys-Ser-Arg, Arg-Sar, Arg-Thr, Asn-Lys-Ser-Arg, Asp-His-Ser-Arg, Asp-Lys-Ile-Arg, Asp-Lys-Lys-Arg, Asp-Lys-Ser-His, Asp-Orn-Ser-Arg, Gly-Asp-Lys-Ser-Arg, Gly-Lys-Val-Arg, Lys-D-Ala-Lys, Lys-Asp, Lys-Glu-Lys, Lys-Leu, Lys-Leu-Lys-NH_2, D-Lys-NH_2, D-Lys-$NHCH_2CH_3$, Lys-D-Phe, D-Lys-Ser,

Lys-Ser-Ala, Lys-Ser-Arg, Lys-Ser-NH_2, Lys-Thr-Arg, Lys-Tyr-Lys-NH_2, Lys-D-Val-Lys-NH_2, N-Me-Lys-D-Ser, Orn-D-Ala, Phe-Lys-D-Ala, Phe-Lys-Sar, Phe-Lys-Ser-Tyr, Sar-D-Lys-Ser, Ser-Lys-Val-Arg, Tyr-Lys-Gly and Val-Lys-Val-Lys-NH_2.

10. A process for the preparation of a peptide of formula I characterized in that such peptide is liberated from its corresponding functional derivative which may be bound by a covalent bond to a solid resin by treatment under acidic or basic conditions, and, if desired, the peptide thus obtained is esterified, amidated and/or acylated to yield a corresponding ester, amide, N- and/or O-acyl derivative or is transformed into one of its pharmacologically acceptable salts by treatment with a base or an acid.

11. A pharmaceutical preparation comprising a peptide of formula I and at least one pharmaceutically acceptable carrier.

12. Use of a peptide of formula I for regulating immune system responses.

13. Use of a peptide of formula I for suppressing autoimmune disease responses.

14. Use of a peptide of formula I for suppressing organ transplantation rejection responses.

15. Use of a peptide of formula I for suppressing neoplastic cell growth.

16. Use of a peptide of formula I for suppressing T-lymphocyte proliferation in mixed lymphocyte response cultures or in antigen-specific T-lymphocyte proliferation assay cultures.

FIGURE 1

NEUROLOGIC DEFICIT SCORE OF SJL/J MICE IMMUNIZED WITH SPINAL CORD HOMOGENATE IN COMPLETE FREUND'S ADJUVANT AND TREATED WITH SALINE OR 1 mg PEPTIDE LYS-SER

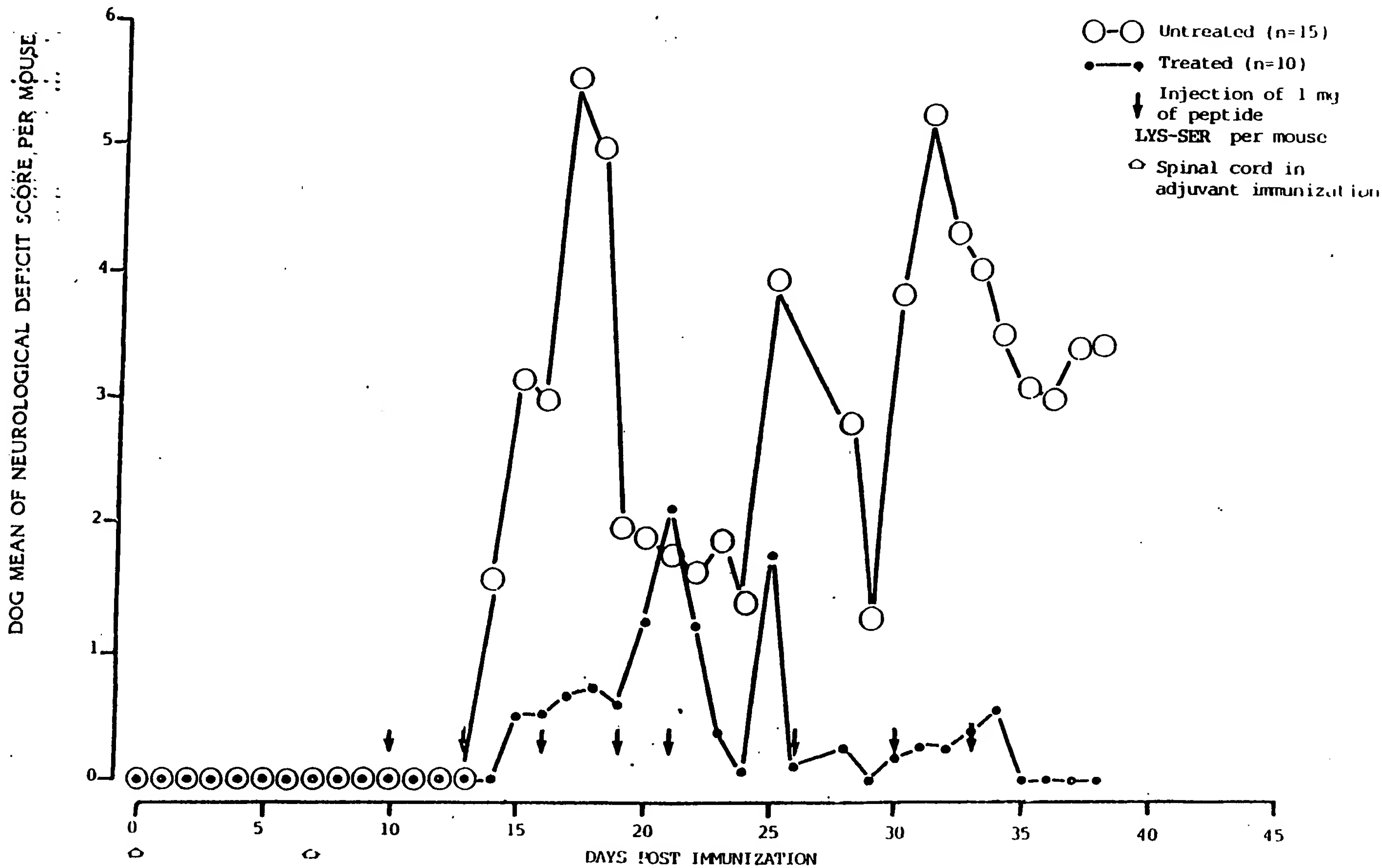
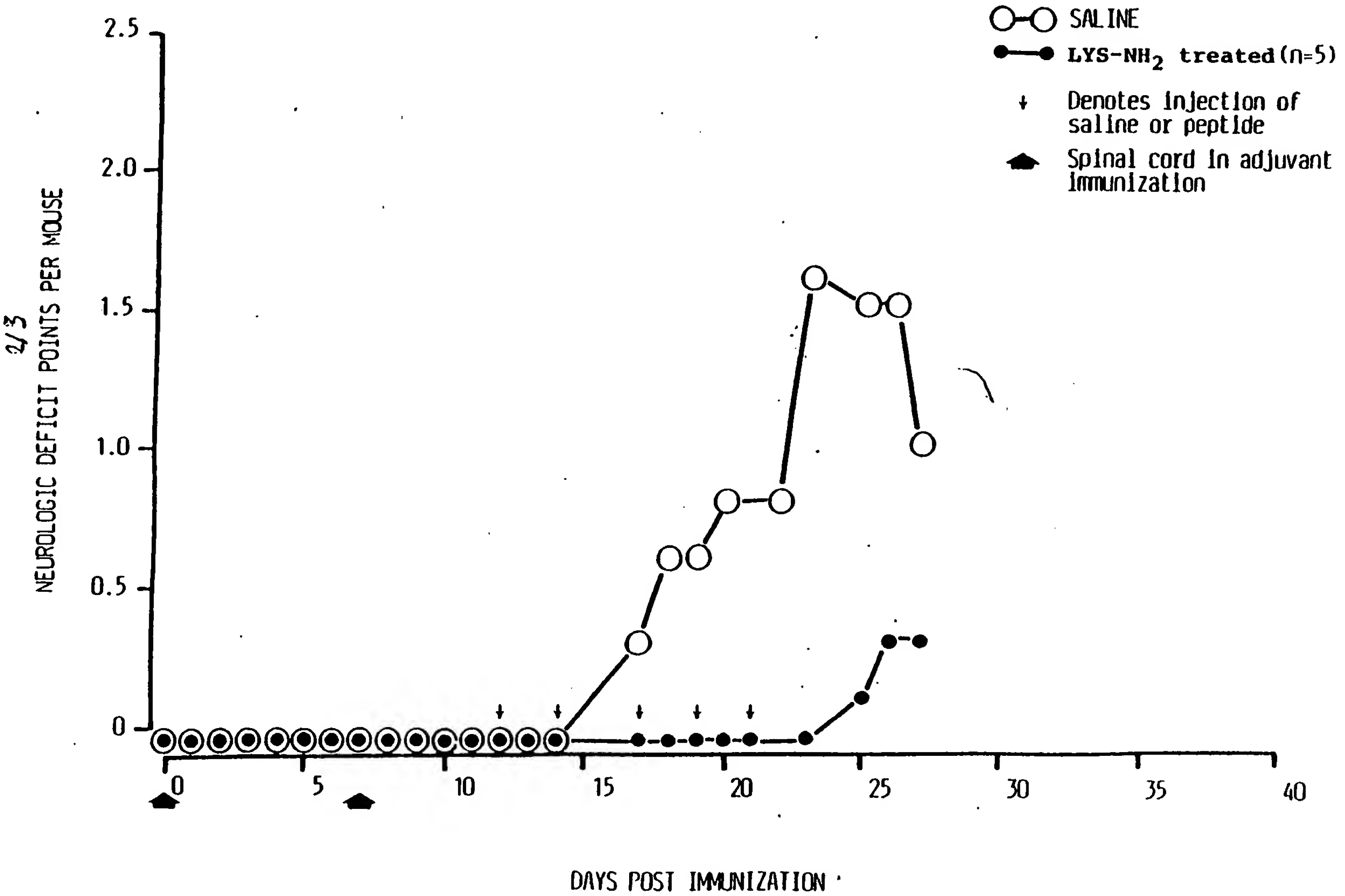


FIGURE 2

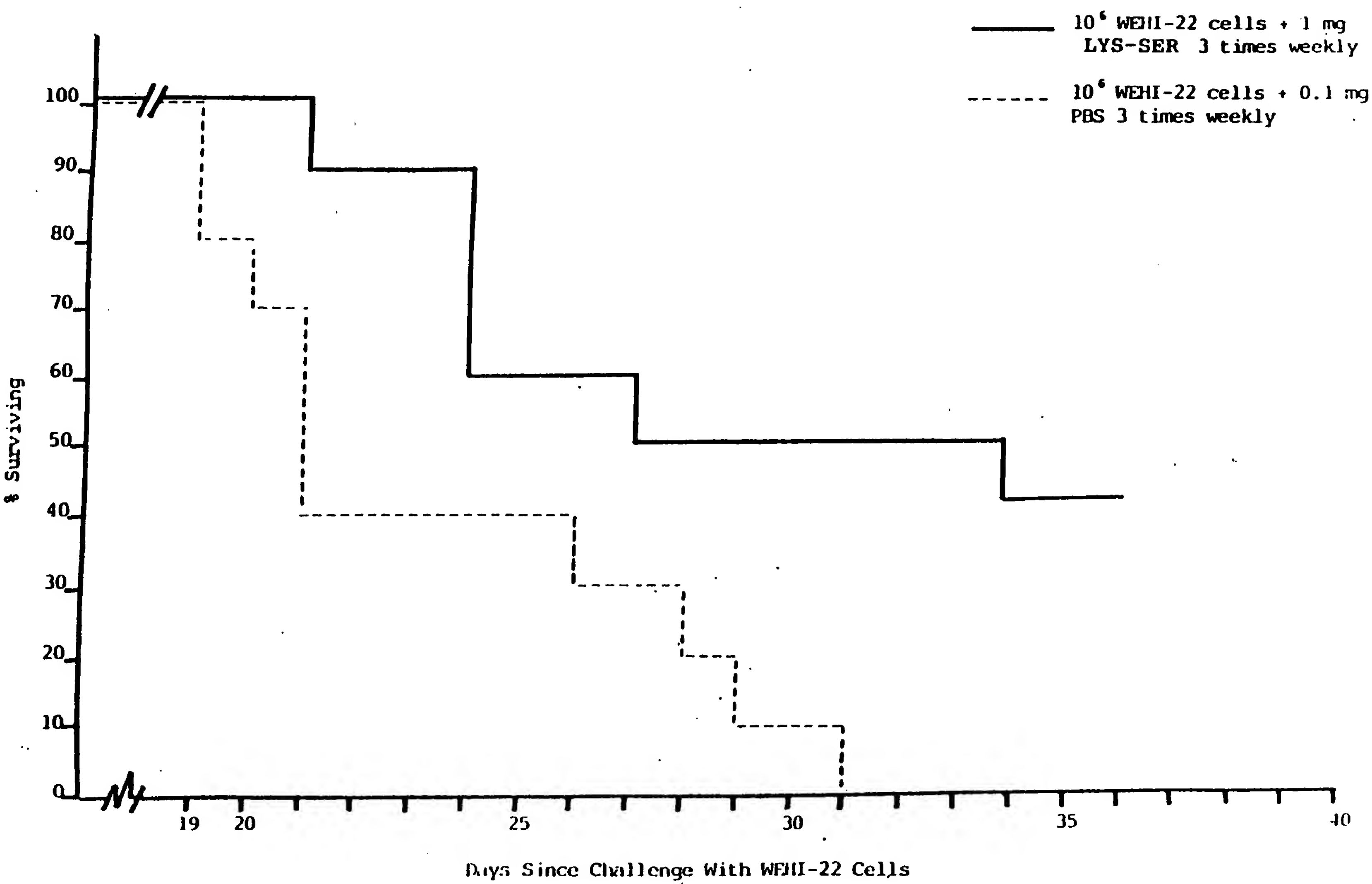
NEUROLOGIC DEFICIT POINTS OF SJL/J MICE IMMUNIZED WITH SPINAL CORD HOMOGENATE IN COMPLETE FREUND'S ADJUVANT AND TREATED 3 TIMES PER WEEK WITH SALINE OR 1 mg PEPTIDE LYS-NH_2



2/3

FIGURE 3

Survival Times of BALB/C Mice Challenged With WEHI-22 Leukemia Cells and Treated With LYS-SER or PBS Three Times Weekly



INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 86/00012

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^4: C 07 K 5/00; C 07 K 7/00; A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^4	C 07 K 5/00 C 07 K 7/00 A 61 K 37/02

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Unlisted Drugs, volume 34, no. 12, 1982 (FK 156), page 182	1,5,8-11
	--	
P,X	Chemical Abstracts, volume 103, no. 9, September 1985, Columbus, Ohio, (US) Cipens G. et al.:"Synthesis of immunopoietins, novel biologically active low-molecular-weight fragments of immunoglobulins G, M, E and A", see page 687, abstract no. 71682e & Bioorg. Khim 1985, 11(4), 437-46	1,5,8-11
	--	
X	Chemical Abstracts, volume 98, no. 21, 1983, Columbus, Ohio, (US) Kawai, Yoshio et al.: "Studies on a new immunoactive peptide, FK-156 III. Structure elucidation", see page 705, abstract no. 179857h & J. Antibiot. 1982, 35(10), 1293-9	1,5,8-11
	--	
X	Chemical Abstracts, volume 97, no. 25, December 1982, Columbus, Ohio, (US) Kitaura, Yoshihiko et al.:"Synthesis and	./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18th June 1986	23 JUL 1986
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

International Application No. PCT/EP 86/00012

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

	immunostimulating activity of FK-156 analogs: fatty acid derivatives of N-(Na-(γ-D-glutamyl)-L-lysyl)-D-alanine", see page 907, abstract no. 216707m & Chem. Pharm. Bull. 1982, 30(8), 3065-8 --	1,5,8-11
X	FR, A, 2485924 (ZAIDAN HOJIN) 8 January 1982, see title page; pages 33-34 --	1,5,8-11
P,X	FR, A, 2549724 (SANYO) 1 February 1985, see the whole document --	1,5,8-11
X	US, A, 3864481 (HASHIM) 4 February 1975,	./.

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers °)...... because they relate to subject matter not required to be searched by this Authority, namely:

°) Claims searched completely : 7-9
Claims searched incompletely : 10-16 (only as far as relating to the compounds of Claims 7-9)
Claims not searched : 1-6 (chemical compounds which are not characterised by their chemical constitution but by their biological activity)

2. ☐ Claim numbers.........., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers.........., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International Application No PCT/EP 86/00012

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	see the whole document --	1,5,8-11
X	US, A, 4390528 (NAJJAR) 28 June 1983, see the whole document --	1,5,8-11
X	US, A, 4442031 (FELIX) 10 April 1984, see the whole document --	1,5,8-11
P,X	DE, A, 3401545 (HOECHST) 14 February 1985, see title page; pages 6-8,12 --	1,5,8-11
P,X	EP, A, 0166612 (ORTHO) 2 January 1986, see the whole document --	1,5,8-11
P,X	EP, A, 0164654 (HOECHST) 18 December 1985, see the whole document --	1,5,8-11
P,X	EP, A,0146266 (ORTHO) 26 June 1985, see the whole document --	1,5,8-11
P,X	EP, A, 0144103 (ORTHO) 12 June 1985, see the whole document --	1,5,8-11
X	EP, A, 0128097 (CNRS) 12 December 1984, see title page; pages 1-3,21 --	1,5,8-11
X	EP, A, 0114787 (CIBA-GEIGY AG) 1 August 1984, see the whole document --	1,5,8-11
X	EP, A, 0080194 (HOECHST) 1 June 1983, see the whole document --	1,5,8-11
X	EP, A, 0056594 (M. PLANCK) 28 July 1982, see the whole document --	1,5,8-11
X	EP, A, 048573 (AMERICAN HOME PRODUCTS CORP.) 31 March 1982, see the whole document --	1,5,8-11
X	EP, A, 0033384 (M. PLANCK) 12 August 1981, see the whole document --	1,5,8-11
X	EP, A, 0016612 (ORTHO), 1 October 1980 see the whole document --	1,5,8-11
X	EP, A, 0016611 (ORTHO) 1 October 1980, see the whole document --	1,5,8-11
Y	Chemical Abstracts, volume 100, no. 19, May 1984, Columbus, Ohio, (US) Konopinska, Danuta et al.:"Synthesis of tuftsinyltuftsin with potential tumoricidal activity", see page 570, abstract no. 156976s & Pol. J. Chem. 1982 (1983), 56(7-8-9), 1063-6	1,5,11
		./.

International Application No. PCT/EP 86/00012

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	EP, A, 0101063 (HOECHST) 22 February 1984, see title page; pages 1-12,24,25-27	1,5,11
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A	GB, A, 2127413 (E. LILLY) 11 April 1984, see the whole document	1,5,11
